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BRANCHING IN THE OPHIOGLOSSACEAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 202

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(WITH PLATES XX AND XXI AND SIX FIGURES)

The occurrence of branching of the rhizome in this family was mentioned by ROEPER (**13**) in 1859, when he described and figured specimens of *Botrychium Lunaria* in which the rhizome bore lateral branches. The origin of such branches was investigated by BRUCHMANN (**2**), who concluded that they were from adventitious buds of superficial origin. FARMER and FREEMAN (**4**) had already ascribed the occasional monopodial branching of *Helminthostachys* to the occurrence of adventitious buds. In 1902, GWYNNE-VAUGHAN (**5**) pointed out that small conical masses of parenchyma occur regularly in the axils of the leaves in this genus and suggested that these are vestigial buds. This view has been confirmed by LANG (**8**), who found that the branches are always axillary in position. The same writer (**9**) has also shown the regular occurrence of similar vestigial buds in the axils of the leaves in *B. Lunaria*, and has demonstrated that the branches of the rhizome arise from these, and not from buds of adventitious origin, as stated by BRUCHMANN.

A branching rhizome of *Ophioglossum vulgatum* was figured by STENZEL (**14**) in 1858; although no statement was made, the figure clearly indicates that the branching is dichotomous. VAN TIEGHEM (**15**) reported similar specimens and, disregarding ROEPER'S figures, stated that all branching of the rhizome in this family is

dichotomous. This view of the character of the branching was confirmed for *O. vulgatum* by POIRAULT (11).

The occurrence of the two methods of branching within this family has suggested the desirability of further examination of this feature. This investigation has accordingly been undertaken with a view to securing further data bearing upon the relation of the three genera to each other and of the family to other Pteridophytes.

Ophioglossum

POIRAULT (11) made the first critical examination of the nature of the branching of *O. vulgatum*. He concluded that the branching is usually only apparent, and that it is due to the development of a stem bud upon a young root before the root has broken through the

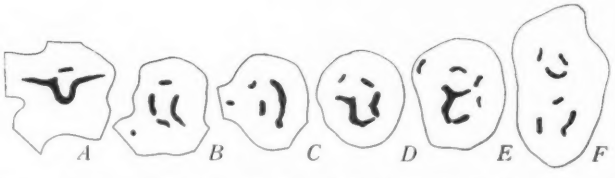

FIG. 1.—Transverse sections of a branching rhizome of *Ophioglossum vulgatum;* only the xylem is shown; ×7.

cortex of the parent rhizome. In a single instance true branching was found, and anatomical examination showed this to be dichotomy. So far as the writer is aware, there is no record of branched rhizomes in other species of this genus.

Ophioglossum vulgatum.—In the examination of some 300 specimens of this species, five branched rhizomes were found. These were examined in serial sections, and in all cases the original stele has divided into two equal and similar steles (fig. 1). There can be no doubt that this is dichotomy, as contrasted with monopodial branching. The term "dichotomy" is here used to denote that branching in which a stem divides into two equal stems, as in *Lycopodium*, and is not meant to imply an exactly equal division of the apical cell of the rhizome.

Ophioglossum pendulum.—In the examination of some 100 rhizomes of this species, two branched specimens were found. As

in *O. vulgatum*, the branching is dichotomous, and the two branches in each case showed an almost exact equality.

In the examination of the material of these two species of *Ophioglossum*, careful search was made for evidence of axillary buds, such as have been described for *Helminthostachys* by GWYNNE-VAUGHAN (**5**), and for *Botrychium Lunaria* by LANG (**9**). No such structures have been found in any case; neither is there any evidence of adventitious budding except upon roots. From this it may be concluded that the true branching of these species is always dichotomous.

Helminthostachys

LANG (**8**) has briefly described the anatomy of two branching rhizomes of this genus. In both, the branches are definitely axillary in position, and it is certain that they have developed from the vestigial buds described by GYWNNE-VAUGHAN (**5**). In each case, the vascular supply of the branch comes from a mass of accessory xylem which develops outside the usual xylem of the stele, either locally at the base of the branch, or surrounding the stele. There is no connection between the branch stele and the subtending leaf trace.[1]

Material of *Helminthostachys* has not been available during the progress of this investigation.

Botrychium

THE OCCURRENCE OF AXILLARY BUDS

All the investigation of branching and of the occurrence of buds has been confined to examination of *B. Lunaria*. On this account, it has seemed advisable to examine other species of this genus with regard to these points. Rhizomes of five species have been secured with branching specimens representing four of these species.

The genus *Botrychium*, as organized by PRANTL (**12**), consists of two sections: EUBOTRYCHIUM, with five species; and PHYLLOTRICHIUM, with eleven species. The latter section is divided into

[1] The full description of this material has just been published (LANG, WM. H., Studies in the morphology and anatomy of the Ophioglossaceae. III. On the anatomy and branching of the rhizome of *Helminthostachys zeylanica*. Ann. Botany **29**:1–54. *pls. 1–3. figs. 1–8.* 1915). In this paper LANG concludes that although no regular cambium is present, "the development of this accessory xylem should rightly come under the conception of secondary thickening."

two subsections: TERNATA, of eight species; and CICUTARIA, of three species. The material investigated consists of rhizomes of *B. ramosum* and *B. lanceolatum* var. *angustisegmentum*, of the section EUBOTRYCHIUM, to which *B. Lunaria* also belongs; of *B. obliquum* and *B. ternatum* var. *intermedium*, of the subsection TERNATA; and of *B. virginianum*, of the subsection CICUTARIA.

Rhizomes of these five species were examined in serial sections for the presence of such axillary buds as have been described by

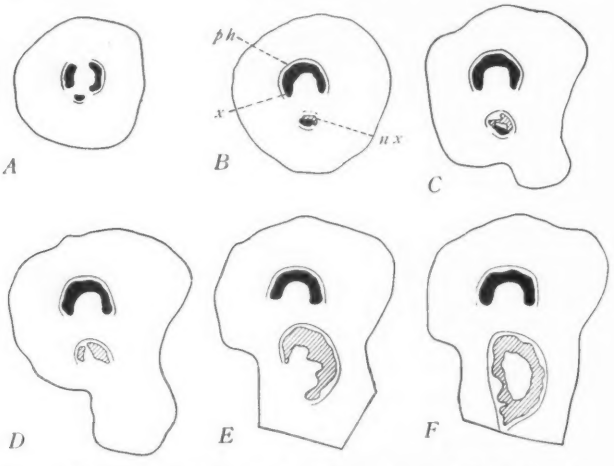

FIG. 2.—Transverse sections of a branching rhizome of *Botrychium lanceolatum* var. *angustisegmentum*: *x*, original xylem of the stem and leaf trace; *nx*, xylem formed after the injury; *ph*, phloem; ×12.

LANG. In all these species, such buds are uniformly present in rhizomes of all ages, and it may be assumed that they are of constant occurrence in all species of the genus. Their origin and development will be considered later.

VASCULAR CONNECTIONS OF THE BRANCHES

ROEPER'S (**13**) figures represent rhizomes of *B. Lunaria* with 2–7 lateral branches. HOLLE (**6**) figured a single branching specimen of the same species in longitudinal section, and pointed out that in this case the branch stele connects with the trace of the leaf

immediately below. LANG (9) has given in detail the vascular connections of the branches in five specimens of this species; he concluded that "the chief vascular supply of the branch is derived from a development of xylem adaxially to the subtending leaf trace." This adaxial xylem is considered to be an extension of the margins of the leaf trace, and therefore centrifugal in character.

Botrychium lanceolatum var. *angustisegmentum*.—A single branching specimen of this species was secured. The terminal bud has been destroyed and the branch has arisen at a considerable distance below. As shown by fig. 2, the vascular system of the branch connects with the subtending leaf trace. After the trace has been separated from the stem stele for a considerable distance, tracheids appear on the adaxial side of the protoxylem of the trace; as shown by fig. 3, these are definitely centripetal in origin and occur in contact with the protoxylem. The mass of xylem formed in this way gives rise to the branch stele, which soon assumes the characters of the main stele. This differs from the general condition in *B. Lunaria*, as described by LANG, in that the branch connection arises from centripetal xylem of the leaf trace rather than from adaxial extension of the centrifugal xylem.

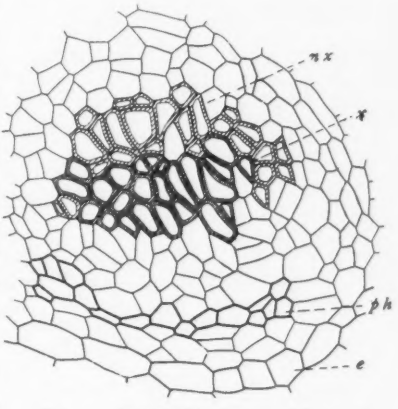

FIG. 3.—Detail of leaf trace of fig. 2, *B*: *e*, endodermis; ×157.

Botrychium ramosum.—Two rhizomes of this species, each bearing a single branch, were secured. In each case the terminal bud has been destroyed and the branch has developed at a considerable distance below. The vascular connections of the branches differ greatly from that described above.

Fig. 4 shows the vascular supply of the branch in the first of these specimens. As the leaf trace swings away from the stem stele, wings of primary and secondary xylem develop in the angle

between the two (figs. 4, *B*, and 7). At first these extend the entire distance between the two and give the appearance of expansion of the stele in the plane of the leaf trace; but later they separate from the stem stele, as shown by fig. 4, *C*. These wings may be considered to be adaxial extensions of the centrifugal xylem of the leaf trace; there is at no point any evidence of centripetal xylem. Soon after the separation from the stem stele, the leaf trace disappears, having been cut off by the abscisss layer and carried out by periderm formation. This leaves two distinct wings of xylem and *each* of these

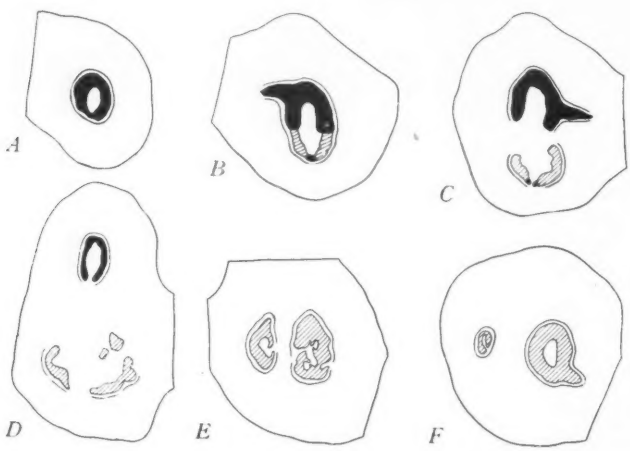

FIG. 4.—Transverse sections of a branching rhizome of *Botrychium ramosum;* ×12.

by extension of its margins rounds up into a definite branch stele, as shown in figs. 4, *D*, *E*, *F*, and 8. One of these branch steles soon disappears, having apparently been unable to meet the competition; its apical region has been entirely obliterated by periderm formation. There can be little doubt that both these branch steles developed from a single axillary bud.

In the other branching specimen of this species, a wing of xylem extends between the leaf trace and the stem stele on one side only. It separates from the stem stele and at a slightly higher point the leaf trace disappears. By extension of its margins, the

wing of xylem rounds up to form a single branch stele. This is exactly the behavior of each of the two wings in the first specimen.

The vascular supply of the branch is here derived in part, at least, from adaxial extension of the centrifugal xylem of the leaf trace, as in *B. Lunaria;* but the formation of either one or two wings of xylem and the consequent formation of one or two branch steles is unique. The significance of this will be discussed later.

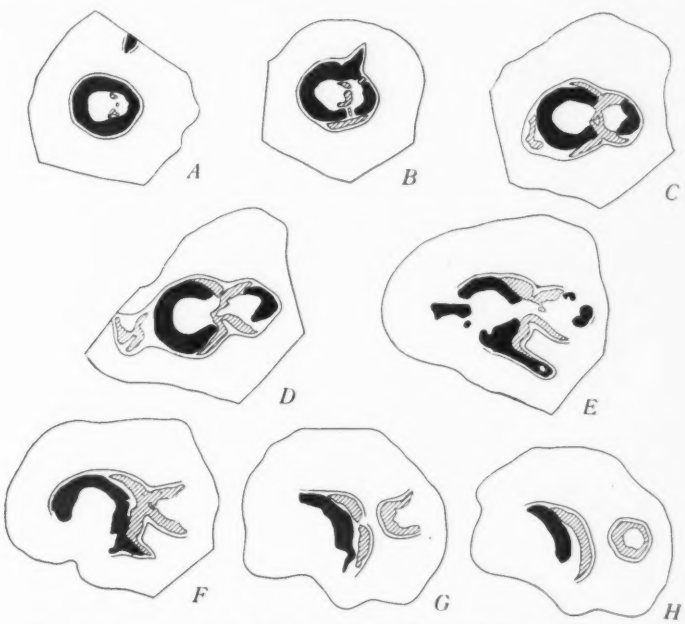

FIG. 5.—Transverse sections of a branching rhizome of *Botrychium virginianum;* ×7.

Botrychium virginianum.—Two branching rhizomes of this species were examined; one of these bore two branches, the other a single one. Fig. 5 shows the vascular connections of the branch in one of these; the injury occurred at the side of the stem and destroyed almost the entire stele (fig. 5, *G*, *H*). As indicated by the figure, there is a development of xylem in the space between the outgoing leaf trace and the stem stele, as in *B. ramosum;* but

here both wings contribute to the formation of a single branch stele. Also procambium strands arise in the pith of the stem opposite the leaf trace (fig. 5, *A*, *B*); these develop a cambium directed toward the center of the stem, which produces a small amount of centripetal secondary wood which also contributes to the vascular supply of the branch. But the greater part of the vascular supply is furnished by the extra or accessory secondary xylem produced by the renewed activity of the cambium (fig. 5, *C*). In this case, therefore, the branch connection consists of (1) accessory secondary xylem, (2) adaxial extensions of the centrifugal xylem of the leaf trace, and (3) a small mass of centripetal secondary xylem originating within the pith of the leaf gap.

One of the other branches shows almost exactly the same connection, but the third branch shows a somewhat different situation. In it the vascular supply is composed principally of accessory secondary xylem; but a small mass of xylem appears in the pericycle of the stem and contributes to the supply of the branch. This pericyclic xylem is added to by a cambium directed toward the phloem; hence we may speak of primary and secondary pericyclic xylem, using the terms "primary" and "secondary" only to indicate the presence or absence of a definite cambium. The vascular supply of this branch, therefore, consists of (1) accessory secondary xylem and (2) primary and secondary pericyclic xylem.

Botrychium obliquum.—Four branching rhizomes of this species were secured; three of these bore two branches each, and the other had three branches. In all but one of these, the apical region had been destroyed; but in this one case the rhizome had been injured at the side below the apical region, but without destroying the entire stele. In all the other cases, the branches developed near the apical region. The vascular connection of the lowest of the three branches mentioned above is shown by fig. 6.

As shown by fig. 6, *A*, a very large development of accessory secondary xylem occurs entirely around the stele before the leaf trace separates; at the same level, a cambium has formed within the pith opposite the leaf trace and has developed a considerable mass of centripetal secondary xylem. The injury which occurred at the side of the stele at a slightly higher level has resulted in the

destruction of the greater portion of the original xylem of the stem; the attendant periderm formation has produced a considerable distortion of the stele. As the leaf trace separates from the stele (fig. 6, *C*) it is surrounded by a ring of xylem composed on the outer side of accessory secondary wood and on the inner side of centripetal secondary wood. This ring splits into two masses (fig. 6, *D*), and the leaf trace is cut off and carried out by periderm

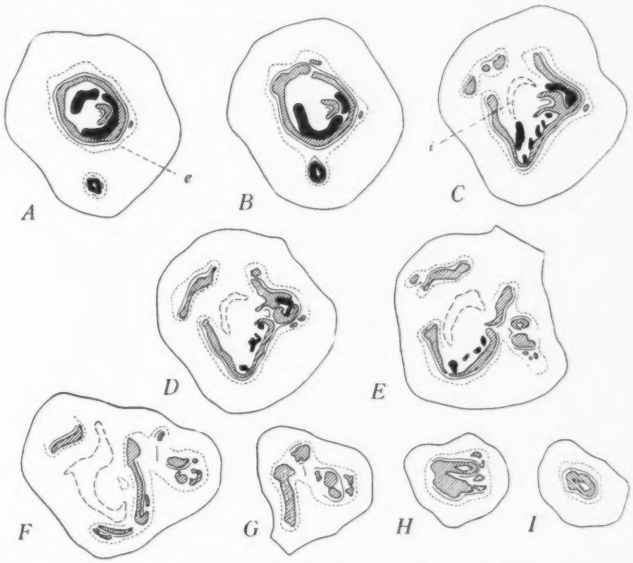

FIG. 6.—Transverse sections of a branching rhizome of *Botrychium obliquum*: *i*, injured region; ×3.5.

formation. At a somewhat higher level, the accessory secondary xylem closes across the gap (fig. 6, *F*).

At a lower level (fig. 6, *E*), masses of xylem arise in the pericycle of one of the strands which formed part of the ring surrounding the leaf trace. Similar masses of pericyclic xylem occur outside the accessory secondary wood which closed the leaf gap (fig. 6, *F*). The development at this point results in the formation of a closed ring of xylem with cambium and phloem on the inside (fig. 6, *G*); at a slightly higher level, this becomes a tangled mass of tracheids

with occasional scattered sieve tubes and parenchyma cells. All these strands—the two produced by the splitting of the ring of xylem about the leaf trace, the strands which arise in the pericycle of one of those, and the tangled mass which is both accessory and pericyclic in origin—fuse to form the branch stele (fig. 6, *H*). Hence the vascular supply of the branch consists of (1) centripetal secondary xylem, (2) accessory secondary xylem, and (3) primary and secondary pericyclic xylem.

In other cases, numerous scattered tracheids appear in the pith below the point of separation of the leaf trace (fig. 9). As the trace leaves the stele, some of these swing out with it and contribute to the vascular supply of the branch. In such cases, there is not the slightest evidence of an internal cambium. Primary centripetal xylem of this kind occurs in the vascular supply of five of the nine branches, while abundant centripetal secondary wood is present in the other four.

It is not necessary to describe in detail the vascular connections of the other branches. Accessory secondary wood usually forms a considerable part of the supply and is present in every case. In six of the nine branches, wings of xylem form between the leaf and the trace and the stele, as in *B. ramosum;* in the other three such formations are entirely lacking. Xylem of pericyclic origin makes up a part of the connection in five cases; in three of these a definite cambium is present. The accessory secondary wood is the only formation which occurs in all the nine cases of this species.

The significance of the wide variation in the vascular supply of the branches will be discussed later.

WOUND REACTIONS

JEFFREY (7) has put forward the view that traumatic reactions are apt to be reversionary in character. BOWER (1) and LANG (9) have used this view in their contention that the pith of the Ophioglossaceae is stelar in character. In the examination of branching specimens of *Botrychium*, especially of *B. obliquum*, some further data upon the nature of the wound reactions of this genus have been secured.

Periderm formation.—The formation of cork at the point of injury has commonly been accepted as a direct response to conditions. In several of the rhizomes of *B. obliquum*, injuries affected all the tissues of the stem. In these cases, periderm is formed by every tissue that is still capable of growth; that is, by cortex, endodermis, pericycle, cambium, and pith. Periderm formation by the pith is shown in fig. 10.

The formation of vascular tissue by the pith.—In three specimens of *B. obliquum*, tracheids scattered through the pith were found in considerable numbers; fig. 9 shows a section through such a stem. In these cases the injury is at some distance above the point of appearance of the pith tracheids.

In both branching specimens of *B. virginianum* and in three of the four of *B. obliquum*, a considerable development of secondary xylem occurs within the pith; figs. 10 and 11 show examples of this. In all cases this development of centripetal secondary xylem occurs below and opposite the point of departure of a leaf trace. The cambium is always directed toward the center of the stele; well developed sieve tubes are present in most cases.

The formation of vascular tissues by the cambium.—In every injured rhizome of *B. virginianum* and *B. obliquum* a renewed activity of the cambium has occurred. Lignification does not always take place in the first elements produced by this renewed activity; this produces a narrow strip of cells of rectangular cross-section just outside the original xylem of the stem. Usually, however, tracheids resembling those of the original growth are produced by this renewed cambial activity (fig. 10); these make up the accessory secondary xylem mentioned above. It is to be noted that no such accessory xylem was found in either of the two species of the section EUBOTRYCHIUM that were examined, but that such formation occurs in *B. Lunaria* as reported by LANG.

The formation of vascular tissues by the pericycle.—In every injured rhizome of *B. virginianum* and *B. obliquum* the pericycle has produced xylem in greater or less amount. As already stated, these masses of pericyclic xylem sometimes contribute to the vascular supply of the branches. When the amount of xylem that occurs is relatively small, only a tangled mass of tracheids is formed; but

frequently a cambium develops and produces tracheids in definite radial rows (fig. 12). The cambium usually occurs on the inner side of the xylem, but in a few cases it is located on the outer side, and sieve tubes occur between it and the endodermis.

The endodermis.—A well marked external endodermis is constantly present in all species of *Botrychium* that were examined. The suberized band on the radial walls is particularly heavy in *B. virginianum* and *B. obliquum*. In uninjured rhizomes of these two species, the external endodermis is continuous except at the points of departure of leaf traces. As a trace swings out from the stele, the endodermis breaks at the side of the trace and closes again rather higher up across the leaf gap; the endodermis on the abaxial side of the trace persists for a short distance only. In injured rhizomes of these two species, the irregular growth of the various stelar tissues has produced distortions and breaks of the endodermal layer (fig. 6). In these specimens, the cells of the endodermis frequently divide by periclinal walls; but there is no evidence that vascular elements are ever formed as a result of this growth. In no case were vascular elements found definitely outside the external endodermis. The only internal endodermis found was that occasionally formed by the folding in of the margins of the external endodermis in cases of greatly disturbed steles (fig. 6, *C*).

Discussion.—The occurrence of scattered tracheids in the pith has been observed in *B. ternatum* by BOWER (1) and in *B. Lunaria* by LANG (9). As stated above, both scattered tracheids and distinct secondary xylem occur frequently in the pith of injured rhizomes of *B. virginianum* and *B. obliquum*. The occurrence of well organized strands of xylem in the pith of a rhizome of *Ophioglossum pendulum* has been reported by the writer (10). In view of these facts, it may be concluded that the pith is definitely stelar in character in *Botrychium* and probably in all three genera of the family.

A slight intrusion of cortical tissue might occur at the point of the break of the endodermis without affecting the pith; such an intrusion would affect only the tissues between the leaf trace and the leaf gap. It may be pointed out that in branching specimens of *B. ramosum*, *B. virginianum*, and *B. obliquum*, xylem is produced

in quantity at this location; hence this tissue is not an intrusion of the cortex, but is stelar in character. It is to be noted that this tissue is opposite a break in the endodermis, but not outside it.

The view is held that there is a morphological distinction between stelar and cortical tissues; that the suberization of the radial walls of a layer of cells is a physiological phenomenon which under the usual conditions of development occurs in the layer of cells next outside the stele; and that this suberization, under the usual conditions, may be considered an indication of the morphological boundary of the stele, but that it is subject to variation with physiological conditions. LANG has concluded that the internal endodermis of *B. Lunaria* is of physiological significance only, and has suggested that its development is associated with the long leaf gaps of the intermediate region of the rhizome. It is to be noted that the apparent internal endodermis in injured specimens of *B. obliquum* (fig. 6, *E*, *F*) occurs between masses of vascular elements and points of injury.

Of the stelar tissues, the tracheids and sieve tubes are incapable of further growth. The remaining tissues are separated by these into three groups: (1) pith, (2) cambium and adjacent parenchyma, and (3) pericycle. The data given above show that all these three tissues may produce vascular elements, either tracheids or sieve tubes. Hence we may conclude that the production of vascular elements by any stelar tissue is limited only by the capacity of the tissue for further growth.

The manner of that further growth of a stelar tissue varies with the species. In *Ophioglossum pendulum*, which has no secondary thickening, the strands of xylem in the pith of an aberrant specimen were primary in origin. In *Helminthostachys*, no secondary xylem is formed under the usual conditions of development; and renewed growth of the stelar tissues, as in the case of branching, produces an irregular secondary thickening of the stele without a definite meristematic layer. On the other hand, *B. virginianum* and *B. obliquum* show very great development of secondary wood under usual conditions. In both these species, renewed growth of the pith, cambium, and pericycle manifested itself in part by the formation of secondary xylem. Hence we may conclude that the

manner of formation of vascular elements by stelar tissues varies with the species, but is relatively constant in any one species under various conditions.

Examination of the various structures shows that they differ mainly in the tissue producing them and in amount of development. That is, these individual variations are differences of position and quantity of vascular elements, and as such their explanation is to be looked for among the physiological factors operating at the time of their development. From this point of view, vascular structures produced as a result of injury may readily show ancestral characters; but such characters are to be considered, not as the repetition of a definite stage of the phylogenetic development of the form, but rather as an indication of the recurrence of certain conditions of development.

It seems well to insist at this point that vascular strands are secondary structures as compared with the tissues which they traverse. The formation of an organ creates a physiological demand to which the vascular strand is a response; and uniformity of the structure which results is only an indication of uniformity of demand and of uniform conditions of development. In this view, the vascular connections of the branches are determined by factors of the same character as those controlling wound reactions.

The vascular supply must be contributed by stelar tissues capable of growth; these tissues are the ones already enumerated, pith, cambium and adjacent parenchyma, and pericycle, together with the parenchyma between the leaf trace and the leaf gap. The manner of formation of the vascular elements of the branch supply is more or less restricted in any species to the particular method of that species. Thus in *B. ramosum*, in which secondary wood formation is relatively slight, no renewed cambial activity occurred in connection with the formation of a branch; while in *B. obliquum*, in which secondary wood formation is very marked under usual conditions, not only does the cambium begin active growth in every case of branching, but similar cambial activity is sometimes set up in both pith and pericycle. The physiological demand likewise varies; in the branch of *B. ramosum* represented in figs. 4 and 8,

two growing points were doubtless established. This produced a physiological demand which, operating under the conditioning factors just described, produced two branch steles.

The conclusion that is reached, therefore, is that the vascular connections of the branches are determined in general by three factors. These factors are (1) the presence of stelar tissues capable of growth within the range of the influence of the developing branch; (2) the nature of the growth which can be induced in those tissues, in particular, whether such growth is cambial or not; (3) the physiological demand produced by the growing branch. The first of these factors will vary with the distance of the bud from the leaf trace as compared with its distance from the stem stele; and, more particularly, with the age of those structures when the branch begins to develop. The second factor may be considered to be relatively constant for any one species but to vary widely with different species. The vascular connection produced is the direct result of the third factor acting upon and limited by the other two; the individual conditions give to this third factor a special value for each particular case; the resultant structures are therefore direct responses to unknown and varying conditions and of physiological interest only. It is therefore concluded that the vascular connections of the branches of *Botrychium* have little or no phylogenetic significance.

THE ORIGIN AND DEVELOPMENT OF THE AXILLARY BUDS

In view of the foregoing conclusion, attention has been turned from the anatomy of the branch connections to the examination of the origin of the axillary buds. Since LANG's description of these buds in *B. Lunaria* deals only with the mature structures, the further investigation has been directed toward the examination of the origin and development of these buds in *B. obliquum*.

The apical region.—The apical region of *Botrychium* has been described in detail by HOLLE (**6**), CAMPBELL (**3**), and BRUCHMANN (**2**). All agree that growth takes place by means of an apical cell of the form of a triangular pyramid, and that each segment of the apical cell probably gives rise to a leaf. BRUCHMANN states that in *B. Lunaria* leaf formation begins by the appearance of an apical

cell within a segment; and that the entire segment rises abruptly above the plane of the apex of the stem.

In connection with the investigation of the origin of the axillary buds, the apical region of *B. obliquum* has been examined, and the results will be given briefly. As shown by figs. 13 and 14, the apical cell is a triangular pyramid, with three cutting faces. The first division of a segment is by a periclinal wall, as stated for *B. virginianum* by CAMPBELL; the further divisions are irregular.

While the limits of the various segments cannot always be exactly defined, it seems certain that each segment gives rise to a leaf; hence one segment is cut off each year. Fig. 13 represents a transverse section through the apical region of a plant collected early in April; the first segment was cut off during the preceding year and has divided transversely. In the second year, irregular divisions, both longitudinal and transverse, take place with the segment; at the end of the year, the segment consists of 6–15 cells. During the third year, this irregular division continues; but there is little or no extension above the plane of the apex. At the beginning of the fourth year, the segment begins a much more rapid growth and rises abruptly above the plane of the apex, as described by BRUCHMANN; at about the same time, an apical cell is recognizable within the segment, and the further growth is definitely apical. It is evident that while the entire segment takes part in the initial growth, the leaf is formed from only a part of the segment; the remainder of the segment builds up the stem tissues. By the end of the fourth year, the leaf has become a hemispherical mass which has grown forward and upward, and its forward margin extends considerably beyond the apical cell of the stem (fig. 14). Early in the fifth year, the fertile spike makes its appearance. It is first recognized as an apical cell on the forward side of the apical cell of the leaf; by the end of the year, it has produced a well defined knoblike structure. During the sixth year, both the fertile and sterile portions of the leaf develop rapidly. In the summer of the seventh year, this leaf breaks through the base of the enveloping older leaf, and the spores are shed in September. The fertile spike then withers, but the sterile portion of the leaf persists

through the winter, and only dies in the early summer of the eighth year, after the emergence of the next younger leaf.

The bud.—As stated above, the first evidence of the formation of a new leaf is the abrupt rise of the entire segment above the plane of the apex. This growth is relatively slight in amount, and the further development of the young leaf is apical. The cells produced by the apical growth are arranged in such definite rows (fig. 15) that it is easy to distinguish the tissue produced by the apical cell.

The axillary bud can first be distinguished on the adaxial face of the base of a young leaf, after the apical growth of the leaf has proceeded to a considerable extent (fig. 15). At this time, it consists of a plate of meristematic cells, 6–8 in number; there is no evidence of an apical cell. The arrangement of the surrounding cells shows clearly that the bud has not come from the apical cell of the leaf, but has arisen from cells carried up by the elongation of the segment. By the upgrowth of the apical region of the stem, this plate of meristematic cells is thrown into a crevice, which lies between the stem and the adaxial face of the base of the leaf; fig. 14 shows the appearance of a bud a year older than that shown by fig. 15. It consists of a mass of 30–40 cells, lying as a plate at the base of the leaf; one of the cells shown has divided by a periclinal wall, but such a division occurs rarely.

In older leaves, the location of this crevice or slit is readily pointed out by the margin of the stipular sheath of the next younger leaf; this makes the identification of the buds in longitudinal sections of the rhizome particularly easy. Fig. 16 shows the margin of the stipular sheath of the functioning leaf of a rhizome, and the bud formed in the axil of the leaf of the preceding year; fig. 17 shows a bud a year older. They consist of plates of cells of a meristematic nature; these plates are never more than two cells in thickness and are usually 5–8 cells in longitudinal extent. In tangential sections of a rhizome (figs. 18, 19), they are seen to be 6–10 cells in width; the canal by which they communicate with the exterior is a mere slit. There is not the slightest evidence of any apical cell, or other indication of an apex. They agree in all essentials with the buds of *B. Lunaria* as described by LANG.

Discussion.—As stated above, neither axillary nor adventitious buds occur in the rhizomes of either *Ophioglossum vulgatum* or *O. pendulum;* and where branching occurs in these species, it is dichotomous. On the other hand, the buds and branching of *Helminthostachys* resemble those of *Botrychium* in every respect. These facts may be considered further evidence of the close relationship of these two genera; at the same time, they will serve to emphasize the differences between these two genera on the one hand and *Ophioglossum* on the other.

LANG (**9**) has mentioned the similarity between the vascular connection of the branches of *Botrychium* and of species of the Hymenophyllaceae. For purposes of comparison, the origin of the axillary buds of a species of *Trichomanes* from Samoa has been examined. The leaf has an apical cell from the beginning and develops to a considerable extent before the branch appears. The formation of the branch is initiated by the appearance of an apical cell. The exact method of formation of this apical cell of the branch was not determined; but it is evident that it arises late and from the growing point of the leaf. The axillary branches of *Trichomanes*, therefore, are foliar in origin. In *Botrychium*, as shown above, the axillary bud is in no way related to the apical cell of the leaf; it arises directly from a portion of a segment of the apical cell of the rhizome; and its position on the base of the leaf is incidental and does not indicate a foliar origin. The similarity between the branching of *Botrychium* and *Trichomanes*, therefore, is not close.

The mature axillary buds of *Botrychium* are of the simplest possible form, an undifferentiated layer of meristematic cells; and it is to be noted that at no time in their development is there any differentiation. This may be accepted as evidence of reduction produced in connection with dormancy; and we may conclude that *Botrychium* and *Helminthostachys* have been derived from a form which branched freely in a monopodial fashion. This is in full agreement with other evidence which points to a relationship of the Ophioglossaceae to the primitive forms of Filicales, especially the Zygopterideae.

Summary

1. Branching of the rhizome of *Ophioglossum vulgatum* and *O. pendulum* is dichotomous; there are no axillary or adventitious buds on the rhizome.

2. Axillary buds are regularly present in five species of *Botrychium*.

3. The vascular connections of the branches in *Botrychium* vary widely with the species and with the individual specimen. It is concluded that the details of the vascular supply of the branch are controlled by the conditions of development and are therefore of little or no phylogenetic importance.

4. In wounded rhizomes of *B. obliquum*, renewed activity of the cambium produces considerable masses of accessory xylem; the pith frequently develops sieve tubes and a cambium which produces secondary xylem in quantity; the pericycle often produces sieve tubes and secondary xylem. It is concluded that in this species any stelar tissues capable of growth may produce vascular elements under the influence of an injury.

5. The axillary bud of *B. obliquum* arises as a plate of meristematic cells on the adaxial face of the base of the very young leaf; it develops without differentiation into a plate of meristematic tissue one or two cells in thickness and 50–60 cells in area, which is buried by overgrowth of surrounding tissue.

6. The data secured is in agreement with the evidence pointing to a relationship of the Ophioglossaceae to the primitive ferns, especially the Zygopterideae.

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The writer wishes to acknowledge the kindness of Professor JOHN M. COULTER in placing the facilities of the Hull Botanical Laboratory at his disposal; and to express his thanks to Dr. W. J.G. LAND for material of *Trichomanes*.

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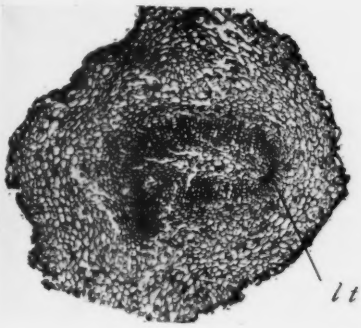
EXPLANATION OF PLATES XX AND XXI

(Figs. 1–6 are in the text)

FIG. 7.—Transverse section of a rhizome of *Botrychium ramosum* at the base of a branch: *lt*, leaf trace; ×20.

FIG. 8.—Transverse section of the branch whose origin is represented by figs. 4 and 7, showing the two branch steles: *a*, larger stele of the branch; *b*, smaller stele of the branch; *r*, trace of root which connects with the smaller branch stele; ×20.



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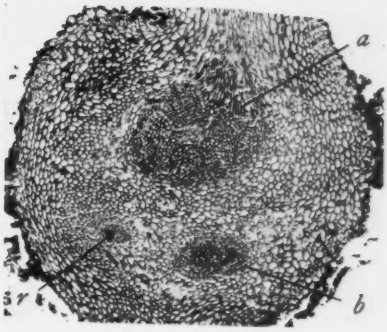

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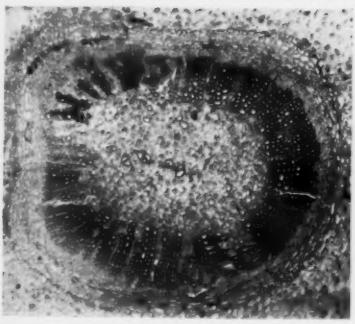

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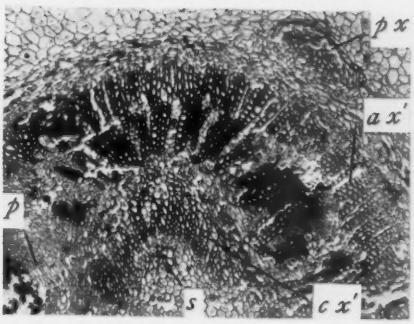

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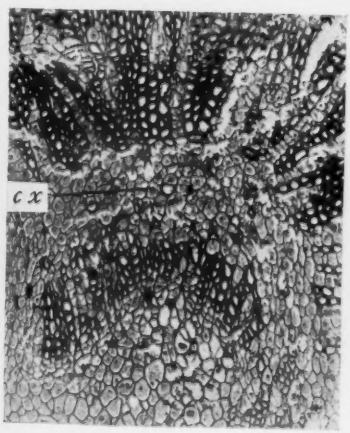

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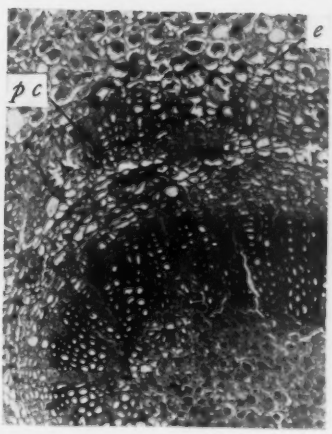

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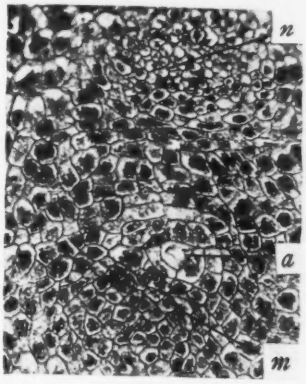
PETRY on OPHIOGLOSSACEAE



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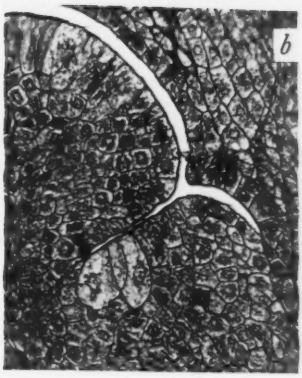

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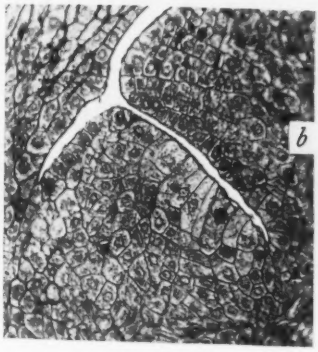

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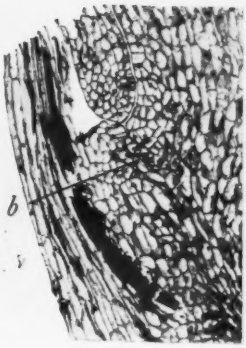

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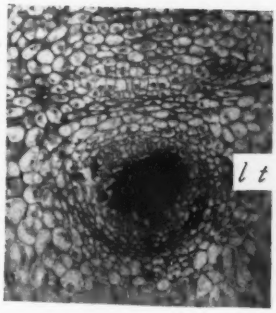

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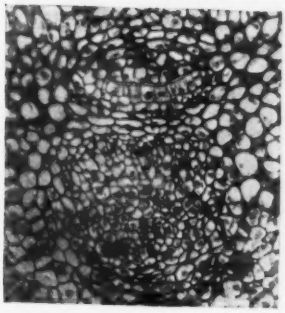

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PETRY on OPHIOGLOSSACEAE

FIG. 9.—Transverse section of a stele of *B. obliquum*, showing tracheids scattered through the pith; ×20.

FIG. 10.—Transverse section of a portion of the stele of an injured rhizome of *B. obliquum*, showing development of new vascular elements in connection with the formation of a branch: *cx'*, centripetal secondary xylem; *ax'*, accessory secondary xylem; *px*, pericyclic xylem; *s*, sieve tubes; *p*, periderm; ×27.

FIG. 11.—Transverse section of a portion of the stele shown in fig. 10, at a lower level, and showing detail of the vascular elements in the pith; *cx*, centripetal primary xylem; ×48.

FIG. 12.—Transverse section of a portion of a stele of *B. virginianum*, showing secondary xylem in the pericycle: *pc*, pericyclic cambium; *e*, endodermis; ×48.

FIG. 13.—Transverse section through the apical region of a rhizome of *B. obliquum: a*, apical cell; *m*, procambium of the youngest leaf; *n*, procambium of the next older leaf; ×122.

FIG. 14.—Longitudinal section through the apical region of a rhizome of *B. obliquum: b*, axillary bud of second leaf; ×122.

FIG. 15.—Longitudinal section through the apical region of the rhizome of *B. obliquum: b*, axillary bud of the youngest leaf; ×122.

FIG. 16.—Longitudinal section through axillary bud of *B. obliquum;* ×48.

FIG. 17.—Longitudinal section of mature bud of *B. obliquum;* ×48.

FIG. 18.—Transverse section of axillary bud near its base, as seen in a tangential section of the rhizome: *ll*, trace of the subtending leaf; ×48.

FIG. 19.—Transverse section of bud near the surface of the rhizome; ×48.

VARIATIONS IN RESPIRATORY ACTIVITY IN RELATION TO SUNLIGHT

H. A. SPOEHR

(WITH TEN FIGURES)

Although the importance of light as a physiological stimulus and as a climatological factor has long been recognized, the complexity and multiplicity of biological light reactions has but recently been realized. As a working hypothesis, I have endeavored to analyze the light reactions by dividing them into two classes: (1) those reactions which are brought about by the light directly inducing chemical or physical changes of certain physiologically important substances *within the organism;* and (2) those reactions which are caused by the light affecting the *environment of the organism.* In the first class fall such reactions as the reduction of the acidity of the plant juices[1] with the consequent effects on growth, the inversion of disaccharides, and a number of other purely photochemical reactions. This paper is a prefatory announcement of a reaction which I believe to belong to the second class. It is my hope, by means of such an analysis, eventually to be able to interpret climatological light reactions upon a sound physiological basis.

As one of the most simply measurable plant activities, I have chosen respiration, as indicated by the evolution of carbon dioxide. The great complexity of the chemistry of this process in no wise affects the results for the present purpose.

The effect of light upon the respiratory activity of living things in general has received considerable attention. Unfortunately, however, in most of these investigations the source and nature of the light used were not considered, and hence we have a mass of uncoordinated and often contradictory results. In 1855 MOLESCHOTT[2]

[1] RICHARDS, H. M., Reports in Yearbook Carnegie Inst. Wash. 1913 and 1914. SPOEHR, H. A., Photochemische Vorgänge bei der diurnalen Entsäurung der Succulenten. Biochem. Zeitschr. **57**:95–111. 1913.

[2] MOLESCHOTT, JAC., Die internationale Sanitäts Konferenz in Rom. Wiener Med. Wochenschr. 1855. nos. 36–38.

noticed the increased carbon dioxide production of the frog under the influence of light, and later the same fact was observed in man by PETTENKOFER and VOIT[3] and others. However, in the higher animals the results become complicated by the action of the light on the nervous system.[4] The experiments on plants have been made mostly with fungi, but on account of the confusing influences of temperature, nutrient media, and sources of light, no definite conclusions can be drawn from these investigations.[5]

Recently, MEYER and DELEANO[6] have found that the carbon dioxide production of leaves at practically constant temperature and in the dark is decidedly higher during the daytime than at night. These workers consider the cause of this variation as lying within the organism. They say:

> Nachdem wir wissen, dass die Aufpraegung einer intermittiernden chronometrischen Bewegungsstruktur, welche zur periodischen Erhoehung der Kohlensaeureproduktion fuehrt, moeglich ist, duerfen wir wohl die Hypothese aufstellen, dass die regelmaessigen Schwankungen der Kohlensaeureproduktion an Stunden des Volltages bei normalen Laubblaettern nur durch den waehrend des Volltages stattfindenden Wechsel der Assimilationsintensitaet und wahrscheinlich erst waehrend ihres individuellen Lebens des Laubblattes hervorgerufen ist.

In the following experiments (mostly with wheat seedlings), the carbon dioxide production in the dark at constant conditions of temperature and humidity was measured by drawing air from out-of-doors over the plants, and then through a standard barium hydroxide solution. It was found that the rate at which carbon dioxide was produced during the hours of daytime was regularly higher than that produced during the night. It is evident that under these experimental conditions the only variable external

[3] PETTENKOFER, M., and VOIT, C., Über Kohlensäureausscheidung und Sauerstoffaufnahme während des Wachens und Schlafens beim gesunden u. kranken Menschen. Sitzber. Akad. Wiss. München **2**:236. 1866.

[4] NEUBERG, C., Beziehungen des Lebens zum Licht. Berlin. 1913 (p. 7).

[5] KOLWITZ, R., Über den Einfluss des Lichtes auf die Athmung der niederen Pilze. Jahrb. Wiss. Bot. **33**:128. 1899.

MAXIMOW, N. A. *Ibid.* Bot. Centralbl. **90**:94. 1902.

[6] MEYER, A., and DELEANO, N. T., Die periodischen Tag- und Nachtschwankungen der Atmungsgroesse im Dunkeln befindlicher Laubblätter und deren vermutliche Bezeihung zur Kohlensäureassimilation. Zeitschr. Bot. **3**:658–701. 1911; **5**:209–320. 1913.

condition to which the plants were exposed was the air of the daytime on the one hand, and that of the night on the other. Are there then any differences in the atmospheric air during day and night which might account for this remarkable variation in the respiratory activity? The first thing to suggest itself is the possible influence of the sunlight on the atmosphere.

The intensity of the violet and ultra-violet rays of the sunlight, as measured at the Desert Laboratory during 18 months, compared with the values generally given for atmospheric ionization, showed a remarkable similarity. DEMBER[7] and others[8] have reported the same fact from observations in other localities. Physicists are not agreed as to the exact relation between sunlight and atmospheric ionization,[9] nor is a discussion of this subject necessary for the present purpose. Suffice it to note that according to the observations of ELSTER and GEITEL, A. GOCKEL, VON SCHWEIDLER, and others, atmospheric ionization exhibits a main maximum about noon, a secondary maximum a little before sunrise, with minima after sunset and before sunrise. These values are subject to certain variations, since the ionization is affected by other meteorological factors, as is mentioned below.

In general, then, the highest respiratory activity takes place during the period of increased ionization. If the respiratory activity and atmospheric ionization are in any way related, the artifical deionization of the air drawn over the plants should have a marked effect on their respiratory activity under the present experimental conditions.

[7] DEMBER, H., Über die ionisierende Wirkung des ultravioletten Sonnenlichts. Physik. Zeitschr. **13**:207–212. 1912.

[8] KAEHLER, K., Luftelektrizität. Leipzig. 1913 (p. 69).

[9] LENARD, P., and RAMSAUER, C., Über die Wirkung Ultravioletten Lichtes auf Gase unter besonderer Berücksichtigung der Vorgänge in der Erdatmosphaere. Metrol. Zeitzchr. **29**:150. 1912.

LENARD, P., Über die Wirkung des Ultravioletten Lichtes auf Gasförmige Körper. Ann. Phys. **1**:486. 1900; **3**:298. 1900.

THOMSON, J. J., Conduction of electricity through gases. Cambridge. 1913 (p. 254).

ELSTER, J., and GEITEL, H., Die Existenz elektrischer Ionen in der Atmosphäre. Jour. Terr. Magnetism and Atmos. Elect. **4**:213. 1899.

STARK, J., Die Elektrizität in Gasen. Leipzig. 1902.

Methods and apparatus

In fig. 1 the apparatus used in this investigation is schematically shown; all of the tubes are of glass with heavy rubber connections. In order to avoid all possible contamination of the air, this was drawn from out-of-doors on the north side of the laboratory building, through a glass tube, and entered the apparatus at E_1. The air was not drawn through any liquid in order to avoid submitting the plants to changes of pressure, and so as not to affect the electrical conditions of the atmosphere. The large bottle (B) contained a 50 per cent aqueous solution of potassium hydroxide; the glass tube was placed so that the air passed immediately over the solution. This bottle was omitted in the experiments of short duration. In order to further remove the carbon dioxide, the air was passed through the bulb tubes (C); the lower portion of the bulbs were filled with 50 per cent potassium hydroxide solution. The tubes were so arranged that they could be shaken from time to time. S represents a glass tube, one inch in diameter, containing coarse soda lime (4 mesh). The air then passed into a Frease electrical thermostat (T). This was kept at 29° in all of the experiments given below. It was, of course, of great importance that the air coming in contact with the plants should also attain the same constant temperature. In order to do this, the air was first passed into a 500 cc. Erlenmeyer flask (F), then through 45 feet of thin-walled glass tubing (G), and finally into the flask R containing the plants. As there was always a small amount of water carried out with the air current, this was condensed and trapped at W. The rate at which the air was allowed to pass through the entire apparatus was controlled by means of the glass stopcock A. It was found that variations in the rate of air from 0.5 to 8 liters per hour caused no difference in the rate of carbon dioxide evolution. P_1 and P_2 represent three-way glass stopcocks, by means of which the course of the air could be changed from one to the other of the Meyer's tubes (M) without interrupting the experiment. The Meyer's tubes were found far more satisfactory for the absorption of carbon dioxide than Pettenkofer tubes or any of the modifications of the latter. Especially was this true in the experiments

running 12–15 hours. The absorption tubes were inserted by making the connection at *L* with heavy rubber tubing; 125cc.

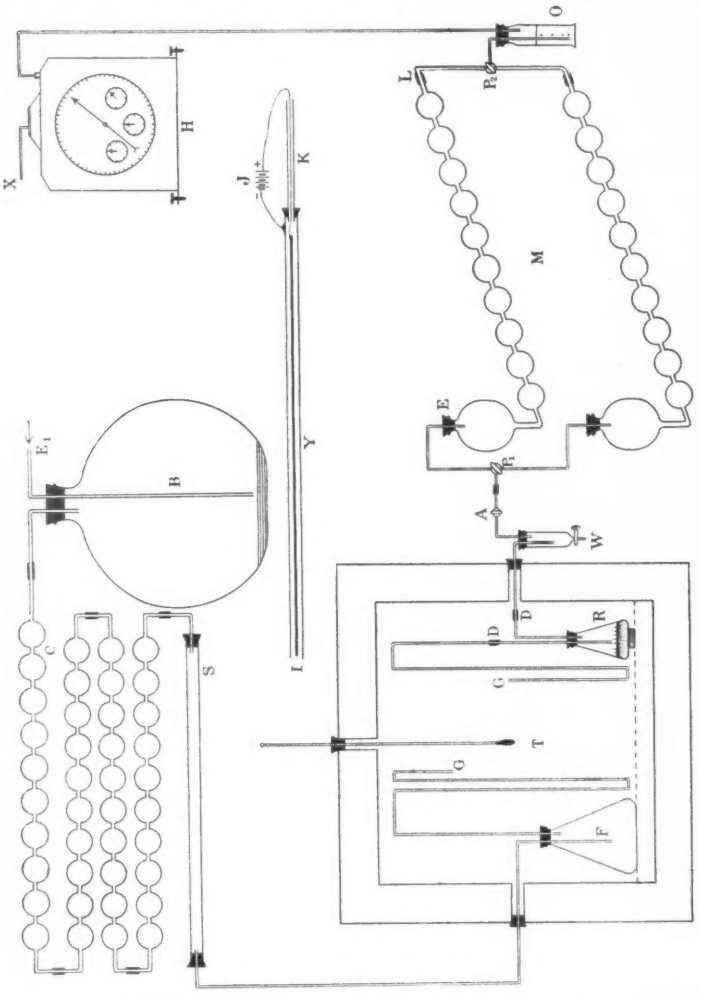

FIG. 1.

of the approximately 0.1 normal barium hydroxide solution was then run into the large bulb, and the connection made at *E* with a

rubber stopper. When the amount of carbon dioxide is large, it is, of course, advisable to use 0.5 normal solution. The tube was then ready for use by turning P_1 and P_2. The error caused by the amount of carbon dioxide of the air in the large bulb was found to be negligible for these experiments. While one tube was in use, the other could be charged. After having drawn the air through one tube for the required length of time, the other was inserted into the system, and the barium hydroxide solution from the first plus the precipitated barium carbonate poured into a narrow bottle, well stoppered, and the barium carbonate allowed to settle for 24 hours; 25cc. of the clear supernatant solution was then titrated with 0.1 normal hydrochloric acid. From the difference between the amount of acid required for the used barium hydroxide solution and the original solution, the amount of carbon dioxide evolved can be very simply calculated. In the figure, *O* is a small wash bottle containing barium hydroxide solution, to detect any escaping carbon dioxide beyond the Meyer's tubes; this was never found to take place. The rate of air flow was measured by means of the gas meter *H*. At *X* connection was made with a Palladin pressure regulator, and this was attached to an electrically driven suction pump.

The nature of the plant material used for these experiments was found to be of greatest importance. Briefly, it is necessary that the organisms be actively respiring, and that the gaseous exchange with the atmosphere be not too difficult. For instance, potato tubers evolve carbon dioxide so slowly that even with a large quantity the differences between day and night are exceedingly slight. There must also be a sufficient supply of carbohydrate food material, for it was found that as soon as the carbohydrates were exhausted, the nature of the carbon dioxide evolution changed greatly.[10] The difficulty with wheat seedlings is, of course, that the relatively rapid rise and fall of the rate of carbon dioxide evolution makes a comparison between day and night

[10] Compare DELEANO, N. T., Untersuchungen über den Atmungsstoffwechsel abgeschnittener Blätter. Jahrb. Wiss. Bot. **51**:541–593. 1912. He found that only after the exhaustion of the carbohydrates was there any evidence of protoplasmic disturbance.

rather difficult. I believe, however, that for the present purpose these experiments will serve as the best illustration.

Before starting an experiment, the wheat grains were always sterilized: *R*, an Erlenmeyer flask (of 100cc. for the experiments with 70 seedlings, 1000cc. when 200 seedlings were used) was provided with a rubber stopper and glass tubing which could be detached at *D*. The bottom of the flask was covered with about an inch of glass wool, and the flask and tubing were then sterilized in the autoclave. The wheat grains were placed in a small cheesecloth sack, immersed for three minutes in a concentrated aqueous solution of chloroform, and shaken to free the seeds from adhering air bubbles. As much as possible of the chloroform solution was removed, and the contents quickly emptied into the sterile flask. Dry, carefully filtered air was then drawn through the flask for at least 24 hours until the seeds were perfectly air dry, so that no trace of chloroform could remain in the flask. Sterile water was then added through one of the tubes; these were then connected at *D*. Material treated in this way very rarely developed any growth of fungus during the course of the experiment, nor did it show any differences in growth as compared with untreated seeds. It is highly improbable, therefore, that the seed coats were penetrated by the chloroform solution. It was found that the wheat could also be sterilized by means of ultra-violet light. This is an exceedingly convenient method, but as it was found that the subsequent growth of the seedlings was somewhat affected, the method was not used for these experiments.

The deionizing apparatus used consisted of a brass tube (*Y*) five feet long and one inch in diameter. Into this was concentrically placed an iron rod, the same length and one-quarter inch in diameter, and held by fiber supports. The tube and rod were attached to the opposite poles of a series of batteries of 50 volts and 800 amperes. To one end of the brass tube was connected a glass tube containing cotton, to act as a filter. The deionizing apparatus was connected at *I* with *E*.

Blank experiments were, of course, always run in order to test the apparatus. In experiments 1, 2, 4, and 5 it will be noted that the night periods are longer than the day periods. It is conceivable

that drawing air through the barium hydroxide solution would evaporate some water, thus make the solution more concentrated, and hence give lower values for the longer periods. The following blank experiment eliminates this possibility.

25cc. original $Ba(OH)_2$ solution = 27.19cc. 0.1 NHCl
25cc. after 3.5 hours = 27.05cc. " "
25cc. after 16 hours = 26.99cc. " "

That differences in atmospheric pressure might account for the daily variation in carbon dioxide evolution also suggested itself. The exceedingly slight variations of pressure at Tucson bear no possible similarity to the values of respiration.

Experimental results

The following is a brief account of a few typical experiments carried out during the winter of 1913–1914. The first column in the tables gives the number of the carbon dioxide determination; the second, the time of day during which it ran; the third, the number of hours; the fourth, the rate per hour at which the air was drawn over the plants in liters; the fifth, the rate of carbon dioxide evolved per hour, expressed in milligrams. In the sixth column the weather is roughly indicated: *A* stands for a perfectly cloudless day; *B*, a few scattered cumulus clouds; *C*, more clouds than *B*, usually thin cirrus; *O*, overcast; and *R*, rain.

The curves are plotted with the rate of carbon dioxide evolution per hour in mg. on the ordinates, the successive determinations on the abscissas; the broken lines indicate the day rates, the solid lines the night rates.

(1) The first experiments (p. 374) were made with small onion bulbs (Australian brown) 0.75–1 inch in diameter. In order to insure ease of gaseous exchange in the onions, the dry outer layers were removed. Previous experiments showed that this operation resulted in but a very slight traumatic effect. After 24 hours, 35 of these small onions were placed in flask *R*. To prevent drying out, the air was drawn over water contained in a bottle inserted between the tubes *G* and the flask *R*. The experiment ran from January 21 to February 2. The results are given in table I and fig. 2. Here it can be seen that the differences in carbon dioxide

TABLE I

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	10:30 A.M.– 5:30 P.M.	7.00	6.86	4.47	A–B
2	5:30 P.M.– 8:30 A.M.	15.00	6.14	3.45	A
3	8:30 A.M.– 5:30 P.M.	9.00	5.78	3.21	C
4	5:30 P.M.– 8:30 A.M.	15.00	5.93	2.57	O
5	8:30 A.M.– 5:00 P.M.	8.50	5.65	2.42	O
6	5:00 P.M.–10:00 A.M.	17.00	5.76	2.13	C
7	10:00 A.M.– 3:15 P.M.	5.25	5.52	2.20	O
8	3:15 P.M.–10:15 A.M.	19.00	5.42	1.98	O
9	10:15 A.M.– 6:15 P.M.	8.00	5.12	1.89	C
10	6:15 P.M.– 9:00 A.M.	14.75	4.95	1.76	O
11	9:00 A.M.– 4:45 P.M.	7.75	4.91	2.11	O
12	4:45 P.M.– 8:30 A.M.	15.75	4.95	1.96	O
13	8:30 A.M.– 5:00 P.M.	8.50	4.82	1.81	O
14	5:00 P.M.– 8:15 A.M.	15.25	4.79	1.67	R
15	8:15 A.M.– 5:15 P.M.	9.00	4.89	1.61	C–A
16	5:15 P.M.– 8:45 A.M.	15.25	4.65	1.58	A
17	8:45 A.M.– 5:30 P.M.	8.75	4.46	1.56	A
18	5:30 P.M.– 8:45 A.M.	15.25	4.39	1.50	A
19	8:45 A.M.– 5:30 P.M.	8.75	4.23	1.67	A
20	5:30 P.M.– 8:30 A.M.	15.00	3.93	1.63	A
21	8:30 A.M.– 5:30 P.M.	9.00	6.22	1.56	A
22	5:30 P.M.– 9:00 A.M.	15.50	6.71	1.45	B
23	9:00 A.M.– 6:00 P.M.	9.00	7.00	1.43	B
24	6:00 P.M.–10:15 A.M.	16.25	6.89	1.56	A

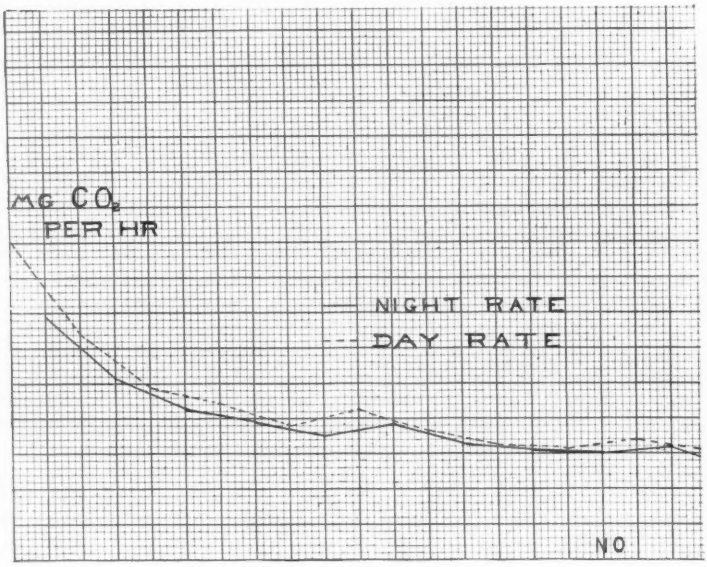

FIG. 2.

production between day and night grow less as the food material is used up, and as the gaseous exchange becomes more difficult, because the shriveled outer layer forms a protecting coat. The amount of carbon dioxide evolved from onions in which the outer layer is dry and contains little food material is very small. $\frac{\text{Day rate}}{\text{Night rate}} = 1.15$.

(2) In the next experiment (p. 376) 75 wheat seedlings were used. This was started after the seedlings were 24 hours old, and ran from February 11 to 24. The results are given in table II and fig. 3. $\frac{\text{Day rate}}{\text{Night rate}} = 1.042$.

(3) In the experiment tabulated on p. 377, 70 wheat seedlings were used, which ran from April 29 to May 9. Several of the seeds were infected with fungus, wherefore the experiment was not run longer. The results are given in table III and fig. 4. $\frac{\text{Day rate}}{\text{Night rate}} = 1.091$.

(4) The deionizing apparatus was used in the next experiment (p. 378), and 70 wheat seedlings were used. The experiment ran from April 14 to April 25. Unfortunately, however, on the night of April 17–18 the pump was out of order, and the determination for that period is not reliable. The results are given in table IV and fig. 5.

(5) Here also (p. 379) the deionizing apparatus was used, with 70 wheat seedlings in the flask. The experiment ran from March 13 to 20. The results are given in table V and fig. 6. $\frac{\text{Day rate}}{\text{Night rate}} = 1.010$.

(6) The deionizing apparatus was used here (p. 380), with 70 wheat seedlings. The experiment ran from May 11 to 22. The results are given in table VI and fig. 7. $\frac{\text{Day rate}}{\text{Night rate}} = 1.015$.

(7) In this experiment (p. 381) the carbon dioxide was determined for three hour periods, and 200 wheat seedlings were used. The experiment ran from May 25 to 27, and the deionizing apparatus was not used. The results are given in table VII and fig. 8. At this time of the year the night time must be considered as falling within

TABLE II

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	5:45 P.M.– 8:45 A.M.	15.00	2.27	2.68	A
2	8:45 A.M.– 5:30 P.M.	8.75	3.09	4.54	A
3	5:30 P.M.– 8:15 A.M.	14.75	4.41	5.59	B
4	8:15 A.M.– 6:00 P.M.	9.75	4.21	6.98	B
5	6:00 P.M.– 9:00 A.M.	15.00	4.40	8.10	B
6	9:00 A.M.– 6:00 P.M.	9.00	4.22	9.20	B–C
7	6:00 P.M.– 9:00 A.M.	15.00	4.13	9.28	C
8	9:00 A.M.– 6:00 P.M.	9.00	3.89	9.50	B–C
9	6:00 P.M.– 9:00 A.M.	15.00	4.00	8.84	O
10	9:00 A.M.– 7:00 P.M.	10.00	2.80	8.67	C
11	7:00 P.M.– 9:30 A.M.	14.50	2.83	8.12	R
12	9:30 A.M.– 7:30 P.M.	10.00	4.90	8.30	R
13	7:30 P.M.– 9:30 A.M.	14.00	5.21	8.16	R
14	9:30 A.M.– 7:30 P.M.	10.00	3.90	8.45	O
15	7:30 P.M.– 9:30 A.M.	14.00	4.00	8.20	R
16	9:30 A.M.– 8:30 P.M.	11.00	3.91	7.64	B
17	8:30 P.M.–10:00 A.M.	13.50	4.08	6.93	B
18	10:00 A.M.– 8:00 P.M.	10.00	3.80	6.60	B
19	8:00 P.M.–10:00 A.M.	14.00	4.14	6.00	B
20	10:00 A.M.– 7:15 P.M.	9.25	4.22	5.70	C
21	7:15 P.M.– 9:15 A.M.	14.00	4.07	5.10	R
22	9:15 A.M.– 6:15 P.M.	9.00	4.00	4.78	B
23	6:15 P.M.– 9:15 A.M.	15.00	4.07	4.20	B
24	9:15 A.M.– 6:00 P.M.	8.75	4.00	3.88	O

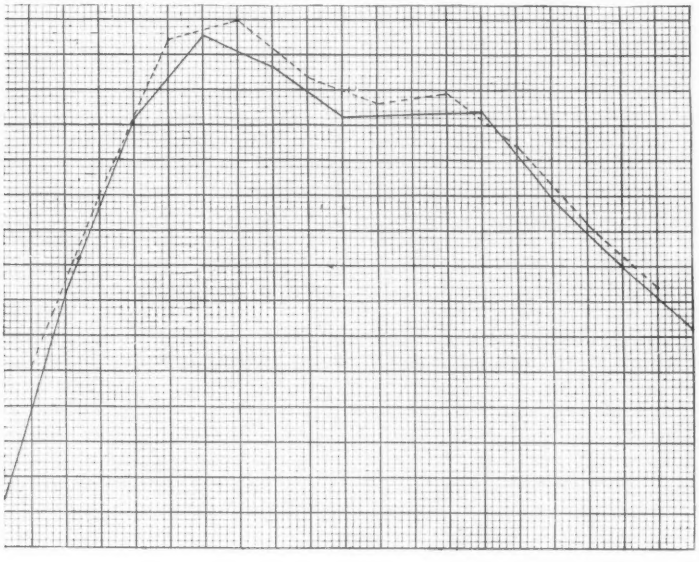

FIG. 3.

TABLE III

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	8:30 P.M.– 8:15 A.M.	11.75	0.76	0.36	B–C
2	8:15 A.M.– 7:45 P.M.	11.50	0.69	0.90	B
3	7:45 P.M.– 8:15 A.M.	12.50	0.96	1.19	B
4	8:15 A.M.– 7:45 P.M.	11.50	1.74	1.76	B–C
5	7:45 P.M.– 8:30 A.M.	12.75	1.88	2.13	B
6	8:30 A.M.– 8:15 P.M.	11.75	1.88	2.62	B–C
7	8:15 P.M.–11:00 A.M.	14.75	2.03	2.97	B
8	11:00 A.M.– 8:45 P.M.	9.75	1.95	3.26	B
9	8:45 P.M.– 8:15 A.M.	11.50	1.83	3.34	A
10	8:15 A.M.– 8:15 P.M.	12.00	1.67	3.46	B
11	8:15 P.M.– 8:30 A.M.	12.25	1.63	3.77	B
12	8:30 A.M.– 8:00 P.M.	11.50	1.83	4.01	O
13	8:00 P.M.– 7:45 A.M.	11.75	1.96	4.16	B
14	7:45 A.M.– 8:00 P.M.	12.25	1.55	4.27	B–A
15	8:00 P.M.– 8:30 A.M.	12.50	1.76	4.40	A
16	8:30 A.M.– 8:00 P.M.	11.50	1.83	4.69	B
17	8:00 P.M.– 8:45 A.M.	12.75	2.12	4.86	B–C
18	8:45 A.M.– 8:00 P.M.	11.75	2.13	5.04	O
19	8:00 P.M.– 8:15 A.M.	12.25	2.28	4.95	C
20	8:15 A.M.– 5:00 P.M.	8.75	2.17	5.06	B

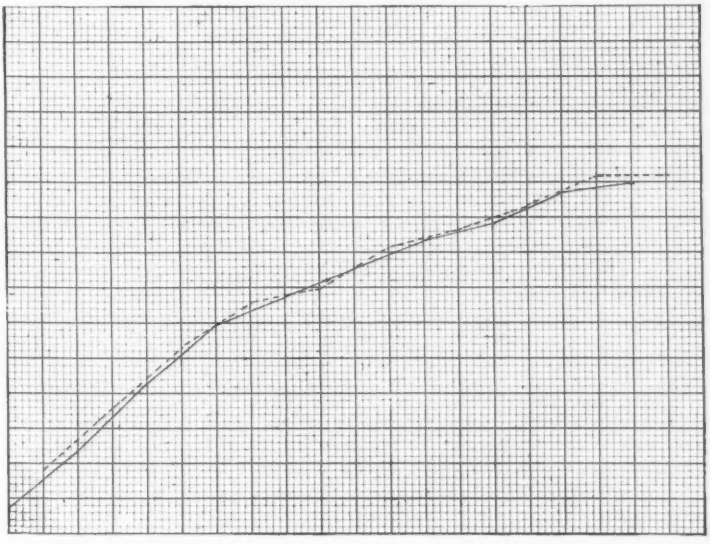

FIG. 4.

TABLE IV

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	4:00 P.M.– 8:30 A.M.	16.50	1.70	0.35	A
2	8:30 A.M.– 5:30 P.M.	9.00	1.33	1.34	A
3	5:30 P.M.– 8:45 A.M.	15.25	1.44	2.73	A
4	8:45 A.M.– 5:30 P.M.	8.75	1.37	3.74	A
5	5:30 P.M.– 9:00 A.M.	15.50	1.37	4.64	A
6	9:00 A.M.– 5:15 P.M.	8.25	1.45	5.80	A
7	5:15 P.M.– 9:30 A.M.	16.25	1.05	.....	A
8	9:30 A.M.– 5:45 P.M.	8.25	1.21	5.99	A
9	5:45 P.M.– 8:00 A.M.	14.25	1.33	7.41	A
10	8:00 A.M.– 6:15 P.M.	10.25	1.17	6.91	A
11	6:15 P.M.– 8:30 A.M.	14.25	1.26	6.60	A
12	8:30 A.M.– 5:30 P.M.	9.00	1.12	6.25	B
13	5:30 P.M.– 8:45 A.M.	15.25	1.18	6.14	O
14	8:45 A.M.– 6:30 P.M.	9.75	1.13	6.18	O
15	6:30 P.M.– 8:30 A.M.	14.00	1.28	6.12	B
16	8:30 A.M.– 6:15 P.M.	9.75	1.13	6.35	B
17	6:15 P.M.– 8:30 A.M.	14.25	1.19	6.23	B–C
18	8:30 A.M.– 5:00 P.M.	8.50	1.06	6.18	C
19	5:00 P.M.– 8:15 A.M.	15.25	1.11	5.74	B–A
20	8:15 A.M.– 5:15 P.M.	9.00	1.00	4.82	A
21	5:15 P.M.– 8:00 A.M.	14.75	0.68	4.67	A
22	8:00 A.M.– 2:45 P.M.	6.75	0.59	4.55	A

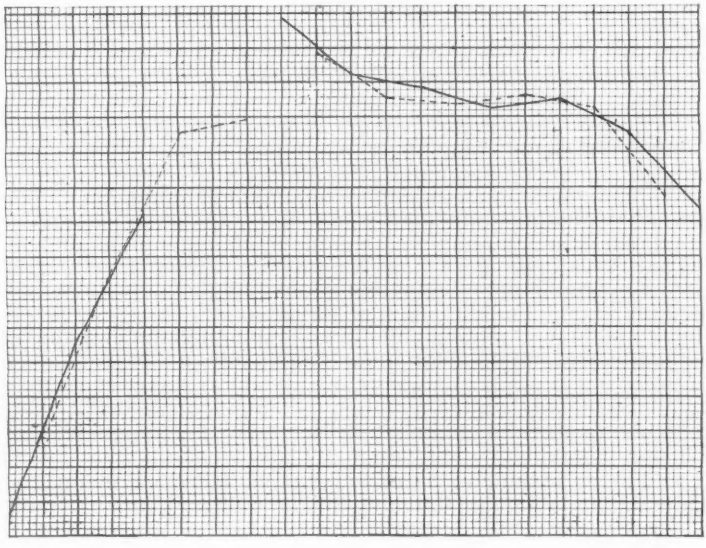

FIG. 5.

TABLE V

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	5:00 P.M.–8:30 A.M.	15.50	3.42	8.14	A
2	8:30 A.M.–6:00 P.M.	9.50	3.90	10.95	A
3	6:00 P.M.–8:30 A.M.	14.50	3.93	12.65	O
4	8:30 A.M.–6:15 P.M.	9.75	4.00	14.81	O
5	6:15 P.M.–8:30 A.M.	14.25	3.93	15.88	A
6	8:30 A.M.–7:30 P.M.	11.00	4.19	16.01	B
7	7:30 P.M.–8:30 A.M.	13.00	4.08	16.45	A
8	8:30 A.M.–5:30 P.M.	9.00	3.11	16.07	B–C
9	5:30 P.M.–8:30 A.M.	15.00	2.93	15.55	O
10	8:30 A.M.–7:30 P.M.	11.00	2.82	15.27	B–C
11	7:30 P.M.–9:00 A.M.	13.50	2.89	14.28	O
12	9:00 A.M.–5:30 P.M.	8.50	3.76	13.14	R
13	5:30 P.M.–8:30 A.M.	15.00	3.87	12.10	B
14	8:30 A.M.–5:30 P.M.	9.00	3.77	11.83	O

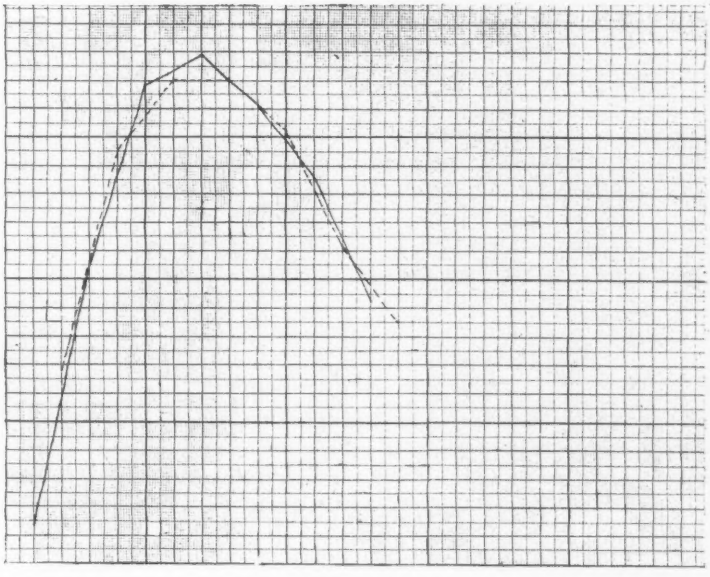

FIG. 6.

TABLE VI

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	9:15 P.M.–8:45 A.M.	11.50	1.65	0.34	C
2	8:45 A.M.–7:00 P.M.	10.25	1.94	0.96	A
3	7:00 P.M.–8:45 A.M.	13.75	1.74	2.03	B
4	8:45 A.M.–8:00 P.M.	11.25	1.91	3.01	O
5	8:00 P.M.–8:15 A.M.	12.25	1.96	3.79	O
6	8:15 A.M.–8:00 P.M.	11.75	1.96	4.56	B
7	8:00 P.M.–8:00 A.M.	12.00	1.83	5.09	A
8	8:00 A.M.–8:45 P.M.	12.75	1.80	5.92	B
9	8:45 P.M.–8:00 A.M.	11.25	1.88	6.20	A
10	8:00 A.M.–8:00 P.M.	12.00	1.93	6.14	O
11	8:00 P.M.–8:00 A.M.	12.00	1.83	6.23	B–A
12	8:00 A.M.–8:00 P.M.	12.00	1.83	6.27	B–A
13	8:00 P.M.–8:15 A.M.	12.25	2.04	5.86	A
14	8:15 A.M.–8:00 P.M.	11.75	1.96	6.11	B
15	8:00 P.M.–8:00 A.M.	12.00	1.93	5.96	A
16	8:00 A.M.–8:45 P.M.	12.75	1.96	5.46	A
17	8:45 P.M.–8:15 A.M.	11.50	2.00	6.65	A
18	8:15 A.M.–8:45 P.M.	12.50	2.18	6.89	B
19	8:45 P.M.–8:30 A.M.	11.75	2.05	7.05	A
20	8:30 A.M.–8:45 P.M.	12.25	2.04	7.31	A
21	8:45 P.M.–8:00 A.M.	11.25	3.70	6.98	B
22	8:00 A.M.–4:15 P.M.	8.25	2.01	5.37	C

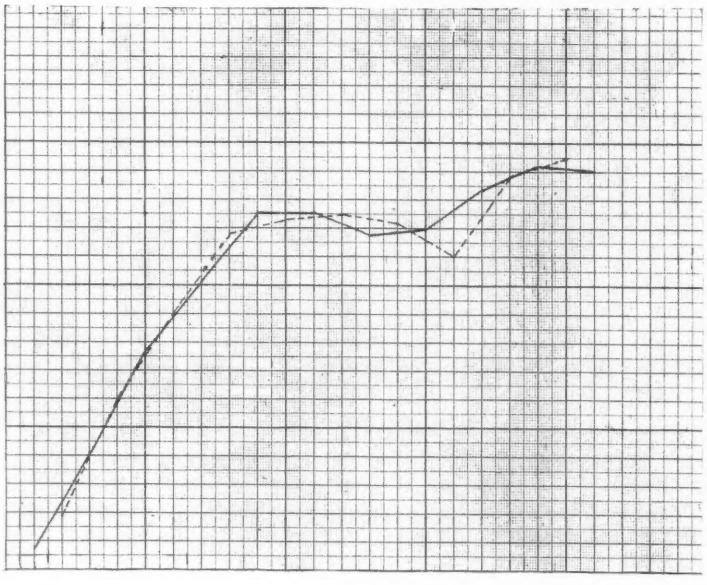

FIG. 7.

TABLE VII

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	12:00 N. – 3:00 A.M.	3	2.00	10.93	A
2	3:00 A.M.– 6:00 A.M.	3	2.33	10.93	A–B
3	6:00 A.M.– 9:00 A.M.	3	2.33	11.88	B
4	9:00 A.M.–12:00 M.	3	2.33	12.47	B
5	12:00 M. – 3:00 P.M.	3	2.00	12.16	B
6	3:00 P.M.– 6:00 P.M.	3	2.33	12.32	B
7	6:00 P.M.– 9:00 P.M.	3	2.33	13.52	B
8	9:00 P.M.–12:00 N.	3	2.33	13.31	A
9	12:00 N. – 3:00 A.M.	3	2.33	14.41	A
10	3:00 A.M.– 6:00 A.M.	3	2.33	14.20	A
11	6:00 A.M.– 9:00 A.M.	3	2.66	15.62	A
12	9:00 A.M.–12:00 M.	3	2.00	15.39	B
13	12:00 M. – 3:00 P.M.	3	2.33	16.01	A
14	3:00 P.M.– 6:00 P.M.	3	2.00	15.91	B
15	6:00 P.M.– 9:00 P.M.	3	2.33	17.11	A
16	9:00 P.M.–12:00 N.	3	2.33	17.22	A
17	12:00 N. – 3:00 A.M.	3	2.00	17.77	A
18	3:00 A.M.– 6:00 A.M.	3	2.00	17.16	A
19	6:00 A.M.– 9:00 A.M.	3	1.67	17.87	A
20	9:00 A.M.–12:00 M.	3	1.67	18.62	A
21	12:00 M. – 3:00 P.M.	3	1.67	19.10	A
22	3:00 P.M.– 6:00 P.M.	3	2.00	18.81	A
23	6:00 P.M.– 9:00 P.M.	3	1.33	19.36	A
24	9:00 P.M.–12:00 N.	3	1.67	18.74	A

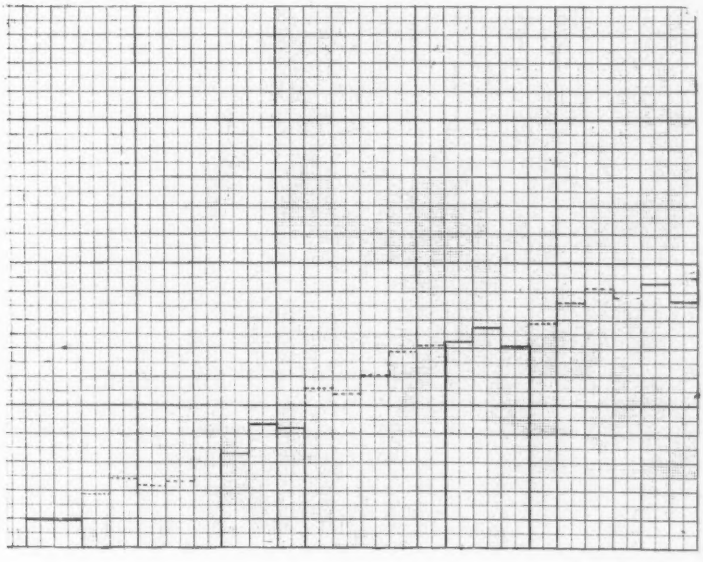

FIG. 8.

TABLE VIII

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	6:00 P.M.– 9:00 P.M.	3	2.67	10.12	B
2	9:00 P.M.–12:00 N.	3	2.00	10.40	B
3	12:00 N. – 3:00 A.M.	3	2.00	11.72	B
4	3:00 A.M.– 6:00 A.M.	3	2.33	12.61	B
5	6:00 A.M.– 9:00 A.M.	3	2.00	13.71	C
6	9:00 A.M.–12:00 M.	3	2.00	14.96	C
7	12:00 M. – 3:00 P.M.	3	2.00	15.72	C
8	3:00 P.M.– 6:00 P.M.	3	2.00	17.00	C
9	6:00 P.M.– 9:00 P.M.	3	2.33	17.30	C
10	9:00 P.M.–12:00 N.	3	2.33	17.60	C
11	12:00 N. – 3:00 A.M.	3	2.33	18.42	B
12	3:00 A.M.– 6:00 A.M.	3	2.33	19.36	B–C
13	6:00 A.M.– 9:00 A.M.	3	2.33	19.68	C
14	9:00 A.M.–12:00 M.	3	2.00	19.91	C
15	12:00 M. – 3:00 P.M.	3	2.33	21.08	R
16	3:00 P.M.– 6:00 P.M.	3	2.00	21.50	R
17	6:00 P.M.– 9:00 P.M.	3	2.00	21.91	R
18	9:00 P.M.–12:00 N.	3	2.33	22.45	B
19	12:00 N. – 3:00 A.M.	3	2.00	23.83	A
20	3:00 A.M.– 6:00 A.M.	3	2.00	25.12	A
21	6:00 A.M.– 9:00 A.M.	3	2.33	25.52	B
22	9:00 A.M.–12:00 M.	3	2.00	26.77	C

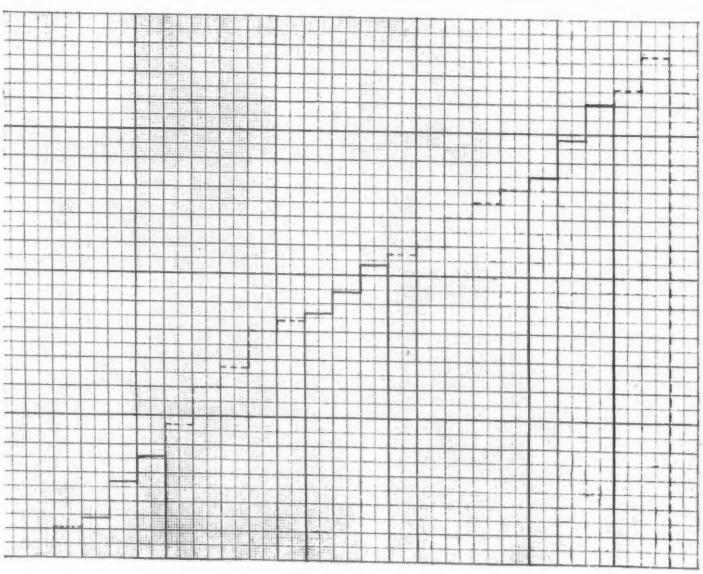

FIG. 9.

the periods between 9 P.M. and 6 A.M. It is impossible to derive an accurate $\frac{\text{day rate}}{\text{night rate}}$ ratio from so short an experiment. An arrangement for automatically taking out the used and putting

TABLE IX

No.	Time	Hours	Air per hour	mg. CO_2 per hour	Weather
1	8:00 P.M.–6:00 A.M.	10.00	4.00	3.36	A
2	6:00 A.M.–8:00 P.M.	14.00	3.70	3.59	A
3	8:00 P.M.–6:00 A.M.	10.00	5.10	3.59	A
4	9:15 A.M.–8:00 P.M.	10.75	2.23	4.18	A
5	8:00 P.M.–6:00 A.M.	10.00	3.30	4.18	A
6	6:00 A.M.–8:00 P.M.	14.00	2.85	4.47	B

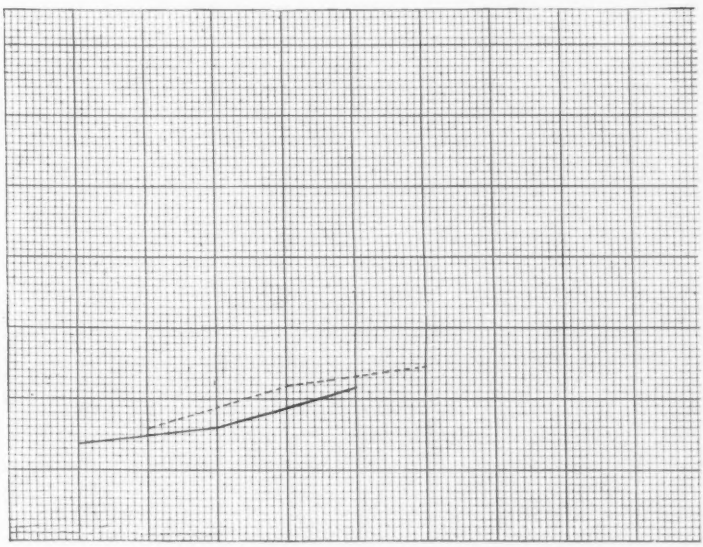

FIG. 10.

in the new Meyer's tubes will materially reduce the personal labor involved in the observations. The striking feature of the curve is the great irregularity of the single observations. Obviously it will be necessary to make measurements of the atmospheric conductivity simultaneously with those of carbon dioxide evolution in order to gain more light on these observations.

(8) Inserting the deionizing tube (p. 382), the other conditions were the same as in 7. The experiment ran from May 28 to 31. The results are given in table VIII and fig. 9. Here the rate shows a very much more regular increase.

(9) In order to determine whether the daily variation in carbon dioxide evolution was restricted to plants or whether it took place also in animals, an experiment (p. 383) was made with ten beetles,[11] placed in flask *R* on fine asbestos fiber soaked in water. Twice during the course of the experiment 2 cc. of water were run onto the asbestos. To all appearances, the beetles behaved perfectly normally during this time. The experiment ran from June 3 to 6. The results are given in table IX and fig. 10. $\frac{\text{Day rate}}{\text{Night rate}} = 1.099$.

Discussion

Although it is difficult to derive an exact $\frac{\text{day rate}}{\text{night rate}}$ ratio with material in which the respiratory activity is rapidly rising or falling, the following in a sense summarizes those of the foregoing experiments which ran for a longer time:

Material	$\frac{\text{Day rate}}{\text{Night rate}}$ normal air	$\frac{\text{Day rate}}{\text{Night rate}}$ deionized air
Onion	1.150	.....
Wheat	1.042	1.010
Wheat	1.091	1.014
Beetles	1.099	.....

There is at present no satisfactory explanation of these facts. The phenomenon of atmospheric ionization is undoubtedly exceedingly complicated, and perhaps at first glance far removed from our present conceptions of climatological factors of physiological importance. However, in view of the fact that ionization of the atmosphere is indicative of important chemical changes in the gases of the atmosphere, a physiological response to these changes is at least to be expected. These chemical changes, no doubt, are of such a nature as to affect the valency or activity of the atmospheric gases, in this case more especially the oxygen.

[11] The material (*Leptinotarsa 10-lineata*) was kindly loaned by Mr. JOHN SINCLAIR, in charge at Tucson of the investigations of Professor W. L. TOWER.

For example, the formation of ozone from molecular oxygen prerequires at least a partial dissociation of the oxygen molecule. It is interesting to note that in Vienna a relation of atmospheric ionization to the amount of ozone was established, the latter increasing with the former.[12] From the work of J. J. THOMSON[13] we know that the chemical effects produced by light are due to the emission of corpuscles from some of the atoms of the illuminated substance. Valency, under this conception, depends upon the relative ability of the atoms to eject or attract corpuscles. Now, it is of especial importance to note that BACH,[14] in elaborating the investigations and theories of MORITZ TRAUBE with especial reference to biological oxidative processes, comes to the conclusion that it is the partially dissociated oxygen (—O—O—) which combines with the oxidizable substance. Furthermore, C. ENGLER,[15] who with his co-workers has done a great deal to extend our knowledge of autoxidation, lays great stress upon the idea that for autoxidative processes the dissociation or liberation of free valencies in the oxygen molecule is necessary, and in this way he explains the accelerating influence of light and heat on oxidative processes. This very brief statement shows, I believe, that the air may possess higher "oxidative power" during the hours of illumination than during darkness.

In the foregoing pages the term respiratory activity has been used in a very general sense, and with special reference to aerobic respiration. There is, of course, little reason for supposing that the ionization of the atmosphere in any way affects the first stages in the katabolic processes, the breaking down or splitting of complex chemical substances. It is probably only in the oxidative processes that the action of the air plays a rôle. In what stages in the series of changes involved in this highly complicated process oxygen

[12] KAEHLER, KARL, Luftelektrizität. p. 56.

[13] THOMSON, J. J., The conduction of electricity through gases. Cambridge. 1913 (p. 290).

[14] BACH, M., Du rôle des peroxydes dans les phénomènes d'oxydation lente. Compt. Rend. Acad. Sci. **124**:951–954. 1897.

[15] ENGLER, C., and WEISBERG, J., Kritische Studien über die Vorgänge der Autoxydation. Braunschweig. 1904.

KASTLE, J. H., The oxidases and other oxygen-catalysts concerned in biological oxidations. U.S. Hygienic Lab. Bull. **59**:9–30. 1910.

enters, is as yet not definitely established; nor can the formation of carbon dioxide be attributed entirely to the oxidative action of the oxygen. We know, however, that the total rate of respiration is greatly influenced by the accumulation or removal of the end products of the first stages in the series. Even assuming, then, that the only function of the oxygen is the removal of these end products, as is maintained by many physiologists, the more rapid oxidation of these substances would result in an increased total rate, and hence increased total amount of carbon dioxide evolved. This higher rate of oxidation, and consequent greater carbon dioxide evolution during the hours of sunlight, could be accounted for by the increased "oxidative power" of the air during this time. That no increased respiratory activity can be obtained with the use of artificial sources of light (except possibly the quartz mercury vapor lamps) is but natural, for light from these sources has no influence on the atmosphere.

The factors influencing atmospheric ionization should be briefly mentioned. Water vapor and high relative humidity have been found to decrease greatly the values; observations made at high altitudes usually show higher values than those made at about sea level. It is to be expected, therefore, that the respiratory activity in the arid and high regions, as, for instance, at Tucson, would be higher and show greater day and night variations than in a moist climate and at sea level.

Finally, it should be stated that the day and night variations as reported in this notice are not of great magnitude, and can be detected only by careful and rather prolonged experiments. The phenomenon is none the less important, however, when considered in its broader physiological bearing. The development of this hypothesis will naturally require many more experiments. Work is now in progress with still finer temperature regulations and on a larger variety of plants and animals. It is my intention to make simultaneous atmospheric conductivity measurements, and to study the effect of air artificially ionized by means of Roentgen rays or radium.

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THE MEDULLARY RAYS OF CEDRUS

M. A. CHRYSLER

(WITH SEVEN FIGURES)

In those conifers the medullary rays of which are provided with marginal cells, it is commonly found that marginal tracheids in the xylem region of a ray are conterminous with the so-called "erect cells" in the phloem region of the ray. Such a condition was figured for *Pinus sylvestris* by STRASBURGER in his notable contributions to the anatomy of the conducting system (**5**), and the figure is copied in his textbook (**6**). It has in fact been claimed by more recent writers (**7**) that the marginal tracheids and the erect cells are corresponding structures, just as are ordinary tracheids and sieve tubes. It was accordingly with some surprise that the writer observed, in the course of a study of the origin of erect cells (**1**), that in the genus *Cedrus* erect cells were conterminous with parenchyma cells in the xylem region of a ray. An insufficient supply of material was at that time available, but recently this lack has been liberally supplied through the kindness of Miss R. HOLDEN of Cambridge University, Mr. R. I. LYNCH, Director of the Cambridge Botanical Garden, Professor E. C. JEFFREY of Harvard University, and Mr. H. N. LEE, formerly of the same institution. From these sources material of all three species of *Cedrus* and of the related monotypic genus *Pseudolarix* has been received, and it is now possible to give an interpretation of the anomalous features of *Cedrus* wood, and to offer evidence as to the relationships of the genus.

In a general way the wood of *Cedrus* bears much resemblance to that of *Abies*, but differs in having more numerous resin cells at the outer edge of annual rings, and in showing ray tracheids, which are, however, mixed with marginal parenchyma. Marginal cells are typically much less abundant than in *Pinus* and other Pineae; in *Cedrus* some rays lack marginal cells, while others are devoid of such cells for considerable distances. STRASBURGER (**5**) has called

attention to the fact that in this genus marginal tracheids occur with uncertainty, and concludes that they are generally more abundant in the mature trunk than in young branches, although they are sometimes scarce in the trunk.

A study of young stems and roots, and of older specimens where the phloem region is preserved, shows that when marginal cells are present in the xylem they are continuous with erect cells in the phloem, but that the latter are frequently present when the xylem

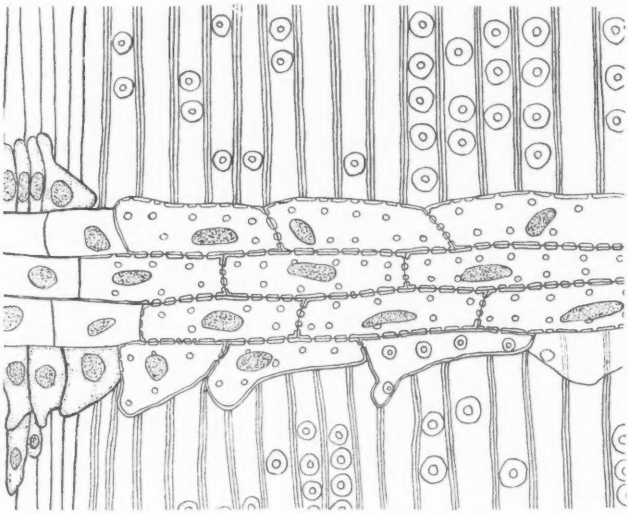

FIG. 1.—*Cedrus deodar*, stem; radial section through twelfth growth ring of xylem, with cambium and a small portion of phloem; in this and the succeeding figures the axis of the stem (or root) lies toward the right; ×290.

has no marginal cells. In such cases a triangular cambial cell occurs at the edge of the ray, as is shown in fig. 1, taken from a twelve-year old branch of *Cedrus deodar*. But in cases where such a medullary ray can be traced through several annual rings, it may generally be made out that scattered marginal cells occur, especially at the end of a year's growth, as may be seen in fig. 2, which represents a continuation of the section shown in fig. 1. Both margins of the ray in question show not only the scattered occur-

rence of the marginal cells, but the mixture of tracheids and parenchyma cells constituting the marginal row. The parenchyma of the marginal rows, like that of the central rows, is more or less completely filled with starch grains, which have been omitted from the figures for the sake of clearness. In some instances the marginal cells are practically restricted to the region of the end of an annual ring, and in such cases the marginal cells are entirely of a parenchymatous nature, as is shown in fig. 3, which is taken from

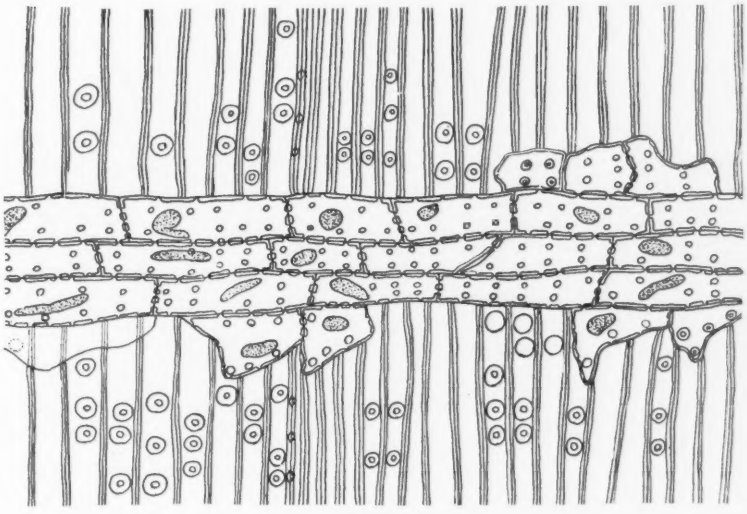

FIG. 2.—*C. deodar*, stem; a continuation of fig. 1, as may be seen from the presence of a shadowy cell on the lower margin of the ray in both figures; ×290.

near the cambial region of a root of *C. libani*. In this and other figures is to be seen the unusual shape of the marginal cells, which are triangular or pointed or tailed. It will be recalled that in most genera which possess marginal cells these are rectangular cells elongated in the radial direction, as are the central cells of a ray.

The lower margin of the ray shown in fig. 2 contains one of the shadowy cells or "ghosts" referred to by THOMPSON (**7**, **8**) as characteristic of *Abies*, and considered by him to represent an

advanced stage of degeneration of marginal cells. These "ghosts" are of very frequent occurrence in the three species of *Cedrus*.

Another common feature of the marginal row is shown in fig. 4, from a root of *C. libani*, where a solitary cell containing rhombic crystals of calcium oxalate appears at the end of an annual ring, a situation which is quite the rule, though not invariable. Crystal-containing cells may also be found among the erect cells of the phloem region of a ray, as is shown in fig. 5. The occurrence of crystals in the phloem parenchyma is universal in *Cedrus*, as in

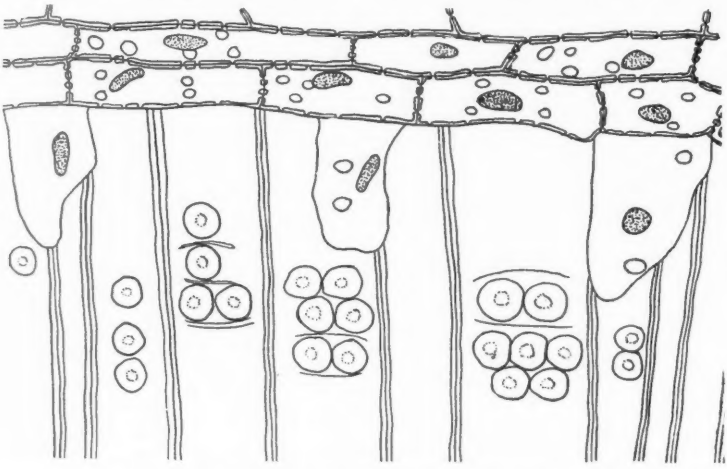

FIG. 3.—*C. libani*, stem; part of a ray traversing three growth rings, at the limit of each of which occurs a marginal parenchyma cell; ×290.

many other genera of conifers. Since calcium oxalate is an end product of katabolism, cells which are charged with it must be regarded as having passed their active or functional stage. This observation together with others here recorded indicate that the marginal cells are in process of degeneration and disappearance.

In order to determine if possible the order of appearance of the structures found at the margin of a ray, serial sections of young stems and roots have been studied. Material of seedlings has not been available, so that the study has been confined to roots and branches of an age of six to seven years. In fig. 6 is shown part of

the fifth and sixth annual rings of a branch of *C. libani*. It will be noticed that on the upper side of the ray the first marginal cell is a tracheid, followed at once by parenchyma, and on the lower side a tracheid is followed by a shadowy cell or "ghost," which still shows a faint bordered pit, and is in turn followed by a row of parenchyma cells. It must be admitted that in some cases the first cells to appear on the margin of a ray are parenchyma cells, but the figure shows the prevailing condition. As far as such observations afford evidence, they indicate that marginal tracheids antedate marginal parenchyma, and where only parenchyma is

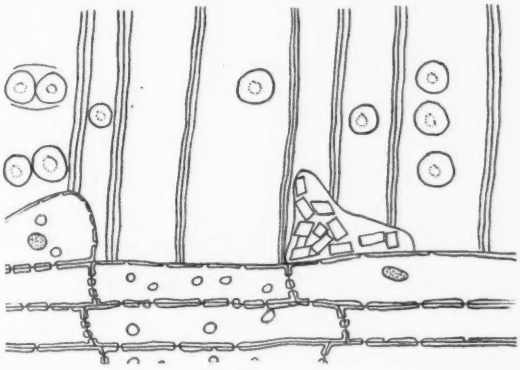

FIG. 4.—*C. libani*, root; a crystal-bearing cell takes the place of starch-bearing parenchyma at the limit of growth ring; ×290.

present it may be inferred that the earlier stages have been passed over.

In a number of cases it has been observed that marginal parenchyma cells are closely associated with resin cells. It will be recalled that in the genus *Cedrus* the resin cells occur on the outer face of the summer wood, and that it is at the limit of an annual ring that marginal parenchyma is most frequent. Fig. 7 shows the close relation of a row of resin cells to parenchyma cells of a ray in a branch of *C. atlantica*. It will be observed that the resin cell in view does not pass behind the ray, but that its end abuts against a marginal parenchyma cell which lies at the outer edge of a layer of summer wood, giving the appearance of a row of resin cells

forming a continuation or outgrowth of a marginal parenchyma cell. Such appearances are fairly common in all three species of *Cedrus*. These observations may throw some light on the origin of the marginal parenchyma. THOMPSON (**8**) in his account of the marginal parenchyma which occurs sparingly in *Abies* suggests that these cells have arisen in connection with the demand for food storage which occurs at the close of the growing season. The close association of marginal parenchyma with resin cells in *Cedrus* suggests, however, that the two structures may have arisen at the same

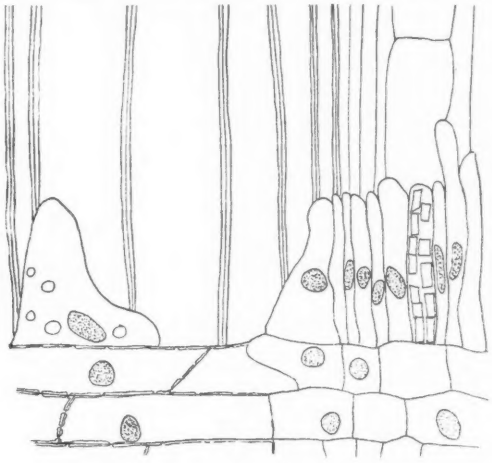

FIG. 5.—*C. libani*, root; one of the erect cells bears crystals; ×290.

time, or even that the vertically elongated marginal parenchyma cells gave rise to rows of resin cells. Of the different positions occupied by resin cells, the terminal position found in *Cedrus* appears to be the original one; at any rate, the plastic materials for supplying such cells are more abundant at the close of the growing season than at any other time. If we consider a marginal cell just cut off from the cambium, it is easy to see that if such cell is in contact with a resin cell which is being supplied with material from the medullary ray, the marginal cell will have a tendency to remain alive rather than thicken its wall and die, that is, develop into a marginal tracheid.

From a number of the observations here recorded the inference seems unavoidable that marginal cells in the genus *Cedrus* are in a vanishing condition. Such observations are: the scattered occurrence of marginal cells, varying from an almost continuous row to occasional cells; persistence of the erect cells of the phloem in line with a few scattering marginal cells on the xylem portion of a ray; the tapering or tailed shape of the cells; occurrence of

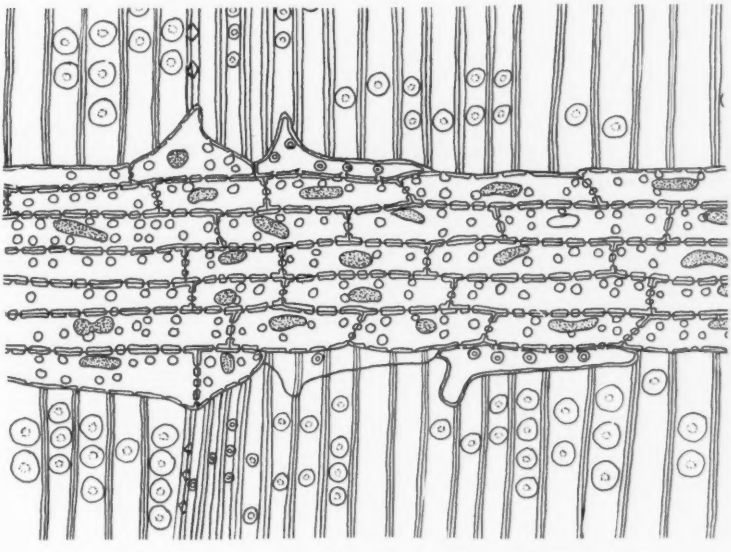

FIG. 6.—*C. libani*, stem; part of the fifth and sixth growth rings, showing earlier appearance of ray tracheids than parenchyma; ×290.

crystal-bearing cells in place of tracheids or starch-bearing parenchyma; occurrence of shadowy cells or "ghosts"; the capricious occurrence of marginal tracheids, as pointed out by STRASBURGER. If then the marginal cells are disappearing, *Cedrus* would appear to show reduction from some such genus as *Pinus* or *Picea*. The foliage of *Cedrus* suggests a relation to *Pinus*, while the cone bears more resemblance to that of *Abies*. The relation to *Pinus* is further indicated by the observation of JEFFREY (2) that when traumatic resin canals are induced in *Cedrus* they occur in both

the vertical and the horizontal plane, while in *Abies* they occur only in the vertical plane. Moreover, resin canals occasionally occur in the xylem of the cone in *Cedrus*, but not in *Abies*.

The foregoing facts go to show that *Cedrus* is in many respects intermediate between *Pinus* and *Abies*. That *Abies* stands farther

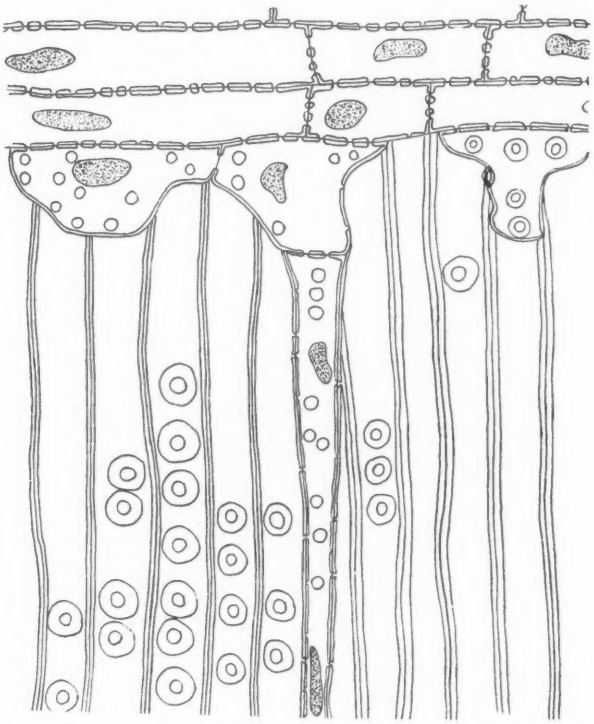

FIG. 7.—*C. atlantica*, stem; at the junction of two growth rings a row of resin cells forms a continuation of a marginal cell of the ray; ×425.

down than *Cedrus* in this reduction series is indicated by the fact that it is mostly in connection with wounds that marginal parenchyma occurs in *Abies*, while such cells occur normally in *Cedrus*. The following evolutionary stages in ray structure are indicated:

1. Medullary rays typically provided with marginal row of cells, which are tracheids: *Pinus*, *Picea*.

2. Marginal row consists of tracheids, but these are replaced by parenchyma at the limit of annual rings: *Cedrus*.

3. Many of the marginal tracheids have been replaced by parenchyma, or have degenerated into ghosts, or have disappeared: *Cedrus*.

4. Marginal cells typically absent, but occur sporadically, especially as a result of injury: *Abies*.

Cedrus shows itself to be a particularly plastic genus, not only in the lack of uniformity of its ray structure, but in its response to wounding, as shown by JEFFREY (2). It would be interesting to ascertain whether wounded material shows reversionary stages in the rays, but my material affords no evidence on this point. In marked contrast to *Cedrus* in this respect is the nearly related *Pseudolarix*. In no part of this plant which has come under my observation has any appearance of ray tracheids been observed, and JEFFREY (*loc. cit.*) remarks upon the absence of wound reactions.

The foregoing observations afford no support to the contention of PENHALLOW that marginal tracheids have been derived from parenchyma. Nor can we agree with his statement (4, p. 107) "the rare occurrence of tracheids in *Thuja*, etc., is to be interpreted as the first evidence of a tendency in development which is only fully realized at a later period." Since these words were written evidence has been accumulating which shows that the series must be read in the opposite direction, and that the sporadic occurrence of ray tracheids in the Cupressineae represents the last stage in disappearance of these cells. The chief evidence in this connection has been supplied by JEFFREY in his study of wound reactions, e.g., in *Cunninghamia* (3) and the observations on the rays of *Cedrus* point in the same direction. Further, there are physiological grounds for opposing the view quoted above, for it is easy to see how a complete row of marginal tracheids can function in carrying water radially, but a few scattered tracheids on the margin of a ray, e.g., of *Thuja*, must be entirely useless, and hence are better regarded as vestigial structures which point back to the time when the ancestors of *Thuja* had a functional row of marginal tracheids. Again, the writer has previously shown (1) that in *Juniperus*, a genus which like *Thuja* shows occasional marginal tracheids, the latter are conterminous with erect cells of the phloem, and erect cells sometimes occur where no marginal tracheids are

to be seen. This appearance is most readily interpreted as a persistence of marginal cells in the phloem after they have disappeared from the xylem.

Summary

1. The medullary rays of *Cedrus* are provided with a margin which varies greatly in composition, being made up of tracheids and parenchyma in varying proportion, or devoid of marginal cells for considerable stretches.

2. Marginal parenchyma when present occurs at the limit of annual rings, and may also extend beyond this point so as to be more plentiful than ray tracheids.

3. The constant occurrence of marginal parenchyma cells at the limit of annual rings, and their close connection with resin cells, indicates that parenchyma has replaced tracheids in connection with secretion of the so-called resin.

4. The marginal cells in *Cedrus* show distinct evidence of being in a degenerating condition.

5. The medullary ray structure confirms the view that *Cedrus* stands intermediate between *Pinus* and *Abies*.

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MICROTECHNICAL METHODS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 203

W. J. G. LAND

An improved method of replacing the paraffin solvent with paraffin

In paraffin imbedding the almost universal custom when replacing a paraffin solvent with paraffin is to add from time to time small pieces of paraffin to the solvent, until at room temperature no more paraffin is dissolved. Then the container is either placed in a low temperature oven or on an oven of higher temperature and paraffin added until the new saturation point is reached. The container is then placed in an oven having a temperature one or two degrees above the melting point of the paraffin and more paraffin is added. When the last paraffin has melted the mixture is poured off and replaced with pure melted paraffin. The object in repeatedly adding small quantities of paraffin is to prevent a too rapid increase in the density of the xylol-paraffin or whatever solvent is used for paraffin.

Objects to be imbedded, being heavier than xylol, sink to the bottom. Paraffin, which is heavier than xylol, also sinks, with the result that the objects are almost immediately surrounded by a dense layer of dissolved paraffin, thereby defeating the end sought by a gradual addition of paraffin. It has repeatedly come to the notice of the writer that much of the damage to delicate plant tissues takes place during the replacement of the solvent by paraffin. Many otherwise careful workers are particularly careless at this point.

In order to prevent the object from coming into immediate contact with the dense xylol-paraffin solution, a piece of wire gauze is bent in such a manner that it forms a support 2–3 cm. above the object, and xylol sufficient to rise 1–2 mm. above the support is added. The container (a shell or bottle) is then filled with blocks

of paraffin, corked, and set aside and not disturbed until the saturation point is reached. The container is next placed on the oven until the new saturation point is reached, next in the oven, and imbedding is proceeded with in the usual manner. Even here, when dealing with delicate plants, the xylol-paraffin is poured off only to the wire gauze and the container again filled with blocks of paraffin.

By this method paraffin is slowly dissolved, and as it descends is slowly and uniformly diffused through the xylol, thus preventing, in a large measure, damage to the object by rapid changes in density. It takes longer to reach the saturation point than when solid paraffin is permitted to fall to the bottom of the container, but little plasmolysis results.

Using delicate liverworts for test objects it was found that no deformation of tissue took place, except that which can be accounted for by the excessively large coefficient of expansion (0.00027854) of paraffin. The deformation caused by paraffin in an artificial cell was found to be exactly the same as is always present in plant cells when the paraffin is quickly cooled.

Many workers use a very close series of alcohols in dehydrating and a similarly close series in replacing alcohol with the paraffin solvent, and then undo all their careful work by indiscriminately adding paraffin to the solvent.

A method of fixing paraffin ribbons to the side with certainty

Albumen fixative, which is almost universally used to fasten paraffin ribbons to the slide, has many excellences and a few disadvantages. Among the latter is the property of coagulating when subjected to moderate heat, and in consequence losing its adhesive quality. Because of this it is sometimes impossible to use sufficient heat to straighten refractory or much wrinkled ribbons, especially if paraffin melting at 58–60° is used. Again, it is almost impossible to fix sections of certain refractory plants to the slide, even if the ribbons are first straightened by floating on warm water and then transferred to an albumen coated slide and allowed to dry without heating. This is particularly true of sections of antheridial and archegonial heads of some mosses and of the strobili of *Selaginella*.

In anatomical work with seedlings and sporelings it is necessary to have an absolutely unbroken series, extending sometimes over many slides, in which the loss of a single section would destroy the value of the entire series.

The well known principle that most colloidal substances, when treated with a solution of some salt of chromium, exposed to light and dried, become insoluble in water, was utilized with complete success. The modern photographic processes, such as printing in pigmented gums and gelatin, photogravure, etc., are based on this property of bichromated colloids.

In the Hull Botanical Laboratory the writer and his students first tried Le Page's liquid glue thinned to the consistency of albumen fixative and made slightly yellow by dissolving a small quantity of potassium bichromate in the thinned glue. The slide was smeared with a thin coating of the bichromated glue and dried in the light. Later a solution of gum arabic was tried with even better results.

The present practice is to spread a few drops of a 1 per cent solution of gum arabic on the slide, taking care to see that every portion of the surface is covered, and flood the slide with water made slightly yellow by dissolving in it a few crystals of potassium bichromate. The ribbons are then straightened out on the slide by means of heat, the excess solution drained off, and the preparation put aside in the light to dry. A very short exposure to light is sufficient to render the gum insoluble in water. After the slides are thoroughly dry they are treated in the usual manner.

In heating the slide to straighten out the ribbons no special precaution, such as necessary with albumen fixative, need be taken, since gum arabic does not lose its adhesive power at temperatures below the melting point of the hardest paraffin ever used in imbedding. The paraffin in the ribbon may even be melted without lessening in the slightest the adhesive property of gum arabic.

When a large number of slides are to be made it is very convenient to mix the gum arabic and the potassium bichromate solutions and flood the slide with the mixture. The solutions should be mixed immediately before using, since the mixture does not keep.

Albumen fixative is much improved if, instead of water, the bichromate solution is used.

In the foregoing process the strength of the bichromate solution seems to be immaterial. If it is stronger than 1 per cent, crystals will appear when the preparation dries. These crystals do no harm, since they never appear in the sections, but they leave unsightly spots on the glass. In practice a 0.2 per cent bichromate solution will be entirely satisfactory. The writer does not make a solution of definite proportions, but adds enough potassium bichromate crystals to make the water pale yellow. A very small quantity of a salt of chromium is sufficient, in the presence of light, to render gum and gelatin insoluble in water.

Imbedding in gelatin

In preparing hard woods for sectioning it is the custom to soften in hydrofluoric acid and imbed in celloidin. Since this process involves dehydration, some refractory woods become unmanageable when sectioning is attempted.

In connection with the work of M. A. BRANNON on the extremely hard stems of plants which had been submerged by the rising waters of the Salton Sea and then exposed when the water receded, a method of imbedding in gelatin was devised by the writer and successfully used. Many of these stems were decorticated, some partially macerated, and all were excessively hard.

Gelatin is soaked in water until no more is taken up, the excess water drained off, and the gelatin liquefied by heat. Pieces of wood previously softened in water, or if necessary in hydrofluoric acid, are placed in the melted gelatin for some hours. Small blocks of hard wood to serve as supports in the microtome are also placed in the melted gelatin. The blocks to be sectioned are properly oriented in a gelatin matrix on the supporting blocks, cooled to set the gelatin, and plunged into strong formalin to harden the gelatin. In cutting the knife is flooded with water.

The advantages of this method are that no dehydration is necessary; that the process is very rapid; and that partly disintegrated tissues are held in place. In careful hands sections of hard woods can be cut as thin as is possible by the celloidin process.

Softening refractory material imbedded in paraffin

Plant material, especially if much starch is present, will not cut readily in paraffin. For complete infiltration with paraffin dehydration must be thorough, and a corresponding hardening of tissues results.

As is well known, paraffin is pervious to water. If imbedded material impossible to cut without fragmentation or tearing of the sections be stored in water for some weeks or months it will in most instances section readily. The effect of water on imbedded material will be most strikingly shown if an attempt is made to cut the gametophyte of some of the cycads at or just after fertilization of the egg immediately after imbedding, and again after the paraffin cakes have lain for some months in water. Dormant embryos of *Helianthus* which will not ribbon immediately after imbedding give unbroken ribbons after the paraffin block has been soaked for some weeks in water. The writer stores in water all paraffin containing hard material.

A method of cleaning cover glass

In attempting to clean cover glasses 50–60 mm. long by wiping with a cloth after they have been freed from the cleaning fluid, many are broken even with the most skilful and practiced handling. Also it is almost impossible to have them free from lint.

In the writer's practice cover glasses are placed in the usual cleaning fluid used for laboratory glassware, a mixture of sulphuric acid and potassium bichromate, rinsed under a tap to completely remove the acid, placed while wet in alcohol, and finally completely submerged in 95 per cent alcohol until wanted. To use, the cover glass is slowly withdrawn from the alcohol so that a minimum film of alcohol will remain on the glass, one end touched to a piece of absorbent paper free from dust to remove the drop of alcohol, touched to a flame, and when the alcohol has completely burned off placed while warm on the slide.

This method is very rapid and gives beautifully clean *cover glasses* with practically no breakage. If the cover glass is drawn from the alcohol so slowly that a very thin film remains, a small crack in the cover glass will not spread.

UNIVERSITY OF CHICAGO

EFFECT OF MOISTURE CONTENT OF A SANDY SOIL ON ITS NITROGEN FIXING POWER

C. B. LIPMAN AND L. T. SHARP

Since attention to the well being of the beneficial soil bacteria is admittedly a vital factor in the maintenance of soil fertility, it becomes as important a question to determine the relation of a soil's moisture content to its bacterial activity as to ascertain the relation thereof to the growth of the plant itself. Relatively little work has thus far been carried out along such lines, and such data as we have bear, so far as we are aware, almost wholly on the relation of the soil's water content to ammonification, nitrification, and dentrification. Nothing but the one investigation below named, which is possessed of any cogency, has thus far come to our notice which deals with the aspects of the same question with regard to nitrogen fixation. The writers therefore present in this paper a series of interesting results bearing on the subject in question which were obtained in the course of some of their studies on the natural nitrogen fixing flora of soils.

The history of the general subject of the relation of soil moisture content to bacterial activity, as above intimated, records but few investigations. Those possessed of any cogency here are those of ENGBERDING,[1] LIPMAN and BROWN,[2] COLEMAN,[3] DEHERAIN,[4] and KRAINSKY.[5] The first named investigator found that the number of bacteria increased with the water content of the soil until the latter reached 80 per cent of saturation, and that it decreased when moisture was supplied in greater quantities. LIPMAN and BROWN found in a neglected clay loam soil that ammonification increased with the increase in water content even up to

[1] Cited from Exp. Sta. Record **21**: 1909, p. 620.

[2] N.J. Exp. Sta. Rpt. 1908, p. 105.

[3] Cent. Bakt. **20**^{2}: 1907–1908, pp. 401 and 484.

[4] Compt. Rend. **125**: 1897, p. 282.

[5] Cent. Bakt. **20**^{2}: 1907–1908, p. 732.

35 per cent of the weight of the soil. They found, however, that nitrification was most active in the same soil with a moisture content of 15 per cent, was only slightly less active with 10 per cent of moisture, and even quite appreciable with 5 per cent of moisture. COLEMAN in working with a loam soil found nitrification in it most active with a moisture content of 16 per cent, thus agreeing with the results of LIPMAN and BROWN. Contrary to the results of the latter, however, COLEMAN obtained marked reduction in nitrification when the moisture content of the soil was reduced to 10 per cent, but again obtained similar results to those of the other investigators when the moisture content of the soil was increased to 26 per cent. DEHERAIN'S findings, in work with the nitrifying flora, were in harmony with those of the foregoing investigations on nitrification. KRAINSKY in working with the nitrogen fixing flora found nitrogen fixation considerable even in soil with less than one-fourth of the optimum moisture content.

In our work on nitrogen fixation a light sandy soil from a walnut grove in Anaheim, California, was employed, and the natural nitrogen fixing flora thereof studied in its relations to moisture. The soil culture method was used in which 50 gram portions of soil were mixed in tumblers with 1 gram of mannite and water added in varying quantities as indicated in the table below. The mixture was stirred with a sterile spatula, the tumblers covered with Petri dish covers and incubated at 28°–30° C. for 21 days. Other explanatory data along with the amount of nitrogen fixed in the cultures with varying quantities of moisture are given below in table I.

It is evident from the figures in the foregoing table that nitrogen fixation in a sandy to sandy loam soil by means of its natural flora and under optimum temperature conditions takes place most actively with a water content varying from 20 to 24 per cent based on the air dry weight of soil, or 22.5 to 26.5 per cent based on the water free soil. Even with 28 per cent of moisture (air dry basis), nitrogen fixation manifests an activity but little less potent than that just mentioned. With a moisture content of 32 per cent a marked decrease in nitrogen fixing power of the soil is evident, and a still greater decrease is noted with the largest water content employed, namely 36 per cent. None the less, it should be noted

that even at the latter moisture content notable nitrogen fixation occurs in what is virtually a saturated soil.

TABLE I

INFLUENCE OF MOISTURE CONTENT ON NITROGEN FIXING POWER OF SANDY SOIL

Culture no.	Per cent moisture air dry basis (hyg. moisture 2.5 per cent)	N found mgs.	N fixed per gram of mannite mgs.	Av. N fixed per gram of mannite mgs.
1	0	31.15	0	0
2	0	31.15	0	0
3	4	31.85	.70	.88
4	4	32.20	1.05	
5	8	34.65	3.50	3.68
6	8	35.00	3.85	
7	12	37.10	5.95	5.95
8	12	37.10	5.95	
9	16	36.75	5.60	5.95
10	16	37.45	6.30	
11	20	39.20	8.05	8.05
12	20	39.20	8.05	
13	24	39.55	8.40	8.05
14	24	38.85	7.70	
15	28	38.15	7.00	7.18
16	28	38.50	7.35	
17	32	36.40	5.25	4.55
18	32	35.00	3.85	
19	36	33.95	2.80	2.98
20	36	34.30	3.15	

With small amounts of moisture in the soil some interesting results are obtained, also, as indicated in the table. Virtually no nitrogen fixation or very little takes place with a moisture content of 4 per cent (air dry basis), but a very marked increase occurs when

8 per cent is present, and amounts of moisture equivalent to 12 per cent (air dry basis) give about the same nitrogen fixation as 32 per cent moisture.

To comment on and summarize the interesting results above given we may make the following statements.

1. Nitrogen fixation by a soil's natural flora being the algebraic sum of the activities of several classes of bacteria both aerobic and anaerobic, it must follow that the greatest fixation of nitrogen will occur at a moisture content very favorable for the most active forms of nitrogen fixing bacteria, and yet not entirely unfavorable for the less potent forms, or vice versa. In the soil in question that point seems to lie between the limits of 20 per cent and 24 per cent of moisture (air dry basis). It would appear just to conclude from these data that the aerobic forms of nitrogen fixing bacteria do best with a 20 per cent moisture content (the optimum for that soil on a physical basis). At higher percentages of moisture up to 24 per cent the anaerobic forms become much more active, while the aerobic forms are depressed in their nitrogen fixing powers. This gives us two maxima of nitrogen fixation in that soil based on the moisture content or what appears from the table to be a curve which runs along at the same plane between rather wide limits.

2. The case of nitrogen fixation with respect to soil moisture content, therefore, would seem to be analogous to that of ammonification as studied by LIPMAN and BROWN as referred to above. In both cases the end products which are measured represent the algebraic sum of the activities of both aerobic and anaerobic organisms.

3. We find in confirmation of the findings of KRAINSKY, above mentioned, that nitrogen fixation is very active even with low moisture content of the soil. Thus, with a moisture content of only 8 per cent very considerable quantities of nitrogen are fixed, and, to judge from the excellent agreement between duplicate nitrogen determinations in our work, appreciable quantities of nitrogen are fixed with a moisture content of only 4 per cent.

4. Taken as a whole, the nitrogen fixing flora of the soil with which we worked, and which may be taken as a criterion for a large variety of sands and sandy loams, behave much more like the

ammonifying flora than like the nitrifying flora with respect to moisture. This is also in harmony with other results from the point of view of factors other than the soil moisture content, which one of us has obtained with respect to the behavior of these three groups of organisms.

5. We feel, as did DEHERAIN in his work cited above, that changes in the physical constitution of a soil will seriously modify the points of maximum and minimum bacterial activity with a given moisture content. But the more exact determinations of available moisture in all soils as advocated by BRIGGS will probably indicate but slight variations from the optimum and minimum moisture contents necessary for the activity of soil organisms as determined for the ammonifying and nitrifying flora by the investigators above named and for the nitrogen fixing flora by us.

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BRIEFER ARTICLES

EVIDENCE FOR THE GENERAL DISTRIBUTION OF OXIDASES IN PLANTS

If the oxidases play the essential rôle in respiration which recent theories have attributed to them, they must be present in all living cells. Two types of tissues, however, have been reported to be without oxidases, namely, tissues markedly acid in reaction and some tissues said to contain large amounts of reducing substances.

Recently it has been pointed out by the writer[1] that in some of the most acid plant tissues, the citrus fruits, the reported absence of oxidases was due to the methods of investigation. By separating out the carpel sacs in such a way that the acid juice and the ferment were kept apart, as is normally the case in the living tissue, good oxidase reactions were obtained; whereas in former methods the tissue was ground in an attempt to extract the ferment, and the latter was then inhibited by the action of the acid.

Tissues reported to be free of oxidases on account of the presence of reducing substances include a few organs of some higher plants and the algae as a group (with but two definite exceptions), so far as they have been investigated.[2]

Examining a few representative forms from the algae with a number of reagents, results as indicated in the accompanying table were obtained. The material was freed from foreign matter as far as possible, ground, or in the case of the large brown forms simply torn up and added to the solutions of the reagent. The formation of oxidation products was then followed by color changes as compared with checks containing boiled material.

The results indicated in the table were further confirmed in the case of the filamentous forms by following the reactions in individual cells. The material was placed in a one-half per cent watery solution of paraphenylenediamine, or equal parts of one-half per cent solution of paraphenylenediamine and alpha napthol (Spitzer's reagent); and in each

[1] Bot. Gaz. **57**:528. 1914.

[2] Atkins, Sci. Proc. Roy. Dublin Soc. **14**:11; Duggar and Davis, Science N.S. **39**:260. 1914.

case sufficient hydrogen peroxide to make the concentration 0.1 per cent. Results were similar in the two cases. In from one-half to ten minutes the oxidation products appeared in the form of minute dark granules exhibiting slow Brownian motion and distributed throughout

TABLE I

THE OXIDATION OF VARIOUS COMPOUNDS BY HYDROGEN PEROXIDE WHEN ACTIVATED BY JUICE EXPRESSED FROM LIVING ALGAE: (+) INDICATES A POSITIVE REACTION IN TEN MINUTES OR LESS; (−) INDICATES NO REACTION EVEN AFTER SOME HOURS OR ONE TOO FAINT TO BE CONSIDERED TRUSTWORTHY.

	Gum guaiac	Pyrogallol	Hydrochinone	Alpha napthol	Alpha napthol and Para-phenylenediamine	Para-phenylenediamine	Alpha napthol and Para-phenylenediamine hydrochloride	Para-phenylenediamine hydrochloride	Benzidine	Aloin	Phenol	Para-cresol	Sodium selenite
Blue-green													
Oscillaria sp.	−	−	−	+	+	+	+	+	−	−	−	−	−
Green													
Conferva sp.	−	−	−	−	+	+	−	−	−	−	−	−	−
Spirogyra sp.	−	faint	faint	−	+	+	+	+	+	−	−	−	−
Ulva sp.	+*	+*	+*	−	+*	+	+	+	+	faint	−	−	−
Enteromorpha sp.	−	−	−	−	+	+	−	−	+	−	−	−	−
Chaetophora sp.	−	−	−	−	+	+	+	+	+	−	−	−	−
Vaucheria sp.	−	−	−	−	+	+	+	+	+	−	−	−	−
Chara sp.	−	−	−	−	+	+	−	−	−	−	−	−	−
Brown													
Laminaria saccharina	−	−	−	−	+	+	+	+	+	−	−	−	−
Fucus sp.	−	−	−	−	+	+	+	+	+	−	−	−	−
Ascophyllum nodosum	−	−	−	−	+	+	+	+	+	−	−	−	−
Red													
Chondrus crispus	−	−	−	−	+	+	−	−	+	−	−	−	−
Polysiphonia sp.	−	−	−	−	+	+	−	−	−	−	−	−	−

* Reaction with and without hydrogen peroxide.

the protoplasm. The size of the granules varied slightly in different species, but in all cases granules were found only in the protoplasm, never in the vacuole. In *Vaucheria* (and occasionally in *Spirogyra*) they showed a tendency to aggregate about the nuclei, though never about the chromatophores. In cells which had been boiled, however,

though the structure of the cell had not been seriously affected, no granules were formed, since the boiling had destroyed the ferment.[3]

An examination of the results summarized in the table shows that, so far as one is able to judge by color changes, the algae as a class possess a ferment capable of activating the oxidation of a limited number of compounds. In other words, it appears that this ferment is specific in its action. The condition is not surprising, inasmuch as it is characteristic, to a greater or less extent, of all ferments and has many parallels among the oxidases themselves.

From the uniformity of results with the forms examined, it is apparent that the oxidases are of general occurence among the algae. These, and the writer's observations on acid tissues, indicate that the oxidases are universally distributed in living plants, and that other cases of their apparent absence may be explained in ways similar to those herein discussed.—G. B. REED, *Laboratory of Plant Physiology, Harvard University.*

[3] A more detailed account of the formation of these granules in plant cells is soon to be published.

CURRENT LITERATURE

BOOK REVIEWS

The Salton Sea

The remarkable overflow of the Colorado River in 1905–1907, causing the submergence of a portion of the Cahuilla Basin, resulted in an expanse of waters known as the Salton Sea. At its maximum, this sea had a depth of 84 feet and an area of 410 square miles, but since the checking of the influx from the Colorado River in 1907, it has suffered an annual subsidence, due to the excess of evaporation over precipitation, averaging about 4.5 feet. These phenomena afforded excellent opportunities for ecological investigations of a unique character, which have been conducted by the Department of Botanical Research of the Carnegie Institution.[1]

The report begins with an account of the discovery, exploration, and geologic history of the Cahuilla Basin, prepared by the late WILLIAM P. BLAKE two years previous to his death in 1910. His connection with the exploration of the area extends from his discovery of the basin in 1853, when he was a member of the Williamson Expedition, to this last visit in 1906. Further details regarding the geographical features of the region are contributed by GODFREY SYKES, who includes reproductions of some of the earliest maps, beginning with one by CASTILLO made in 1541 and first published in 1770. E. E. FREE gives a sketch of the geology and a discussion of the two types of soil, the coarser composed of gravel and sand resulting from the decomposition of the granitic rocks *in situ;* the other, an alluvium of fine texture. Both are decidedly fertile, except for the local development of alkaline conditions. ROSS and VINSON provide a comparison of the chemical composition of the water at various intervals from 1906 to 1913, showing a close resemblance to that of ordinary sea water and an increasing concentration of salts with the present continuous recession. The behavior of micro-organisms in the brine is reported by G. J. PEIRCE, a small red chromogenic bacillus receiving particular attention. M. A. BRANNON, working at the Botanical Laboratory of the University of Chicago upon the action of the Salton Sea water on vegetative tissues, reports no evidence of petrifaction of woody tissues, but a decortication of woody plants submerged for a year or more, due to the enzymic action of bacterial organisms upon the tissues of the cambium region.

[1] MACDOUGAL, D. T., and COLLABORATORS, The Salton Sea. A study of the geography, the geology, the floristics, and the ecology of a desert basin. Carnegie Inst. Pub. 193. pp. 182. *figs. 4. pls. 32.* 1914.

Naturally, the floristics and plant ecology of the area receive the major portion of attention. S. B. PARISH sketches the history of its botanical exploration, from a botanical paper by Dr. C. C. PARRY in Emory's Report of the Survey of the boundary between the United States and Mexico, made in 1856. He presents an annotated list of indigenous and introduced species, the former including 8 trees, 23 shrubs, 10 suffrutescent plants, 30 perennial herbs, and 51 annuals. Only 7 species are endemic. A grouping is made into formations and associations, the halophytic and xerophytic naturally being the most prominent. Detailed sketches of some of the outskirts of the area have already appeared.[2] This analysis of the composition of the vegetation is continued in MACDOUGAL'S inquiry into its genesis, as shown by its re-establishment upon areas sterilized by submergence. He considers both the re-occupation of the strand left bare by the receding lake and that of sterilized islands emerging from the lowering waters. The changes as the aridity of the strand increases, the agencies effective in carrying seeds, and the invasion of new species are among the topics receiving attention, while a detailed history is given of various portions of the strands emerging from the waters. Among the pioneer forms, species of *Atriplex*, *Heliotropium*, *Sesuvium*, *Pluchea*, *Distichlis*, and *Suaeda*, together with *Prosopis pubescens*, *P. glaudulosa*, and *Salix nigra*, are found, but their abundance and survival differ at different points along the shore, and this could be to some extent related to the slope and character of the soil. The fact that 4 out of 60 species found upon the strand showed modifications of structure not observed elsewhere suggests the possibility that the changing conditions are resulting in the production of new species, and that similar series of changes in the past have been similarly productive.

The exactness of the present report and the abundance of its data also combine to make it a most valuable record for the future study of these as well as of other problems which may arise with the continual subsidence of the sea and the further development of the surrounding vegetation.—GEO. D. FULLER.

MINOR NOTICES

Flora of the Dutch West Indian Islands.—BOLDINGH[3] has published a second volume under the foregoing title, which deals with the islands of Curaçao, Aruba, and Bonaire. The present volume is divided into three parts, first Systematical, second Historical, and third Phytogeographical. The last part is subdivided into the following sections: (A) Orological, Geological, and Meteorological, (B) Distribution of the wild plants enumerated in the first part, (C) The vegetation of Curaçao, Aruba, and Bonaire. To this is added an

[2] PARISH, S. B., Sketches of the Colorado Desert. Plant World **17**:122–130. 1914.

[3] BOLDINGH, I., The flora of the Dutch West Indian Islands. Second Volume. Curaçao, Aruba, and Bonaire. 8vo. xiv+197. *pls.* 9. *map* 1. Leyden: E. J. Brill. 1914.

alphabetical index to vernacular and their corresponding scientific names. The three islands represent an area of 860 square kilometers, or about 332 square miles, and possess a flora of 394 species distributed among 80 families from Polypodiaceae to Compositae. Those families predominating, as shown by the number of species recorded, are Leguminosae (41), Gramineae (35), Convovulaceae (25), Euphorbiaceae (24), Cyperaceae (23), and Compositae (20). One new species is described in each of the following genera: *Schizachyrium*, *Ficus*, *Pisonia*, *Kallstroemia*, *Bursera*, *Phyllanthus*, *Croton*, *Maytenus*, *Condalea*, *Casearia*, and *Melampodium*.—J. M. GREENMAN.

Applied botany.—KRAEMER[4] has accomplished a very laborious task for the benefit of students in technical schools, pharmaceutical and medical colleges, food analysists, etc. Although emphasizing the technical side of plants, he has included a basis of morphology and physiology, which should put the student, interested chiefly in the commercial aspect of plants, in touch with the scientific aspect. The seven chapters include the following subjects: Principal groups of plants, under which is given an outline of the plant kingdom; Cell contents and forms of cells; Outer and inner morphology of the higher plants; Botanical nomenclature, which is also a glossary of technical terms; Classification of angiosperms yielding economic products; Classification of medicinal plants; and Microscopic technique of reagents.

The book is a thesaurus of information, and as a book of reference should be of great service to botanists in general.—J.M.C.

North American flora.—The first part of volume 34 presents 50 genera of the Helenieae, all but 2 of them by RYDBERG.[5] The new genera proposed are *Nesothamnus* (type species, *Peritylе incana*), *Leptopharynx* (type species, *Peritylе Parryi*), *Pappothrix* (type species, *Laphamia rupestris*), *Amauriopsis* (type species, *Amauria dissecta*), *Cephalobembix* (type species, *Schkuhria neomexicana*), *Trichymenia* (type species, *Hymenothrix Wrightii*). New species are described also in *Venegazia*, *Psilostrophe* (3), *Baileya* (3), *Peritylе* (5), *Laphamia* (2), *Loxothysanus*, *Bahia* (2), *Hulsea* (3), *Tetracarpum* (2), *Hymenopappus* (5), *Othake* (2), *Rigiopappus*, and *Chaenactis* (3).—J. M. C.

NOTES FOR STUDENTS

Toxic effects.—The observation that small traces of salicylic acid (o-oxybenzoic acid) in the presence of comparatively large quantitites of p-oxybenzoic acid have a deleterious effect on the growth of *Penicillium*, while both p-oxybenzoic and m-oxybenzoic acids serve as food, has led BOESEKEN and

[4] KRAEMER, HENRY, Applied and economic botany. 8vo. vi+806. *figs. 424.* Philadelphia (145 N. 10th St.): Published by the author. 1914. $5.00.

[5] North American Flora **34**: part 1. pp. 80. Carduaceae (Helenieae), by P. A. RYDBERG; *Baeria* and *Lasthenia*, by H. M. HALL. New York Botanical Garden, 1914.

WATERMAN[6] to investigate the behavior of these acids and numerous other compounds with reference to their toxic action and to their nutritive value. The substances investigated cover a wide range, including acids, alcohols, and chlorine and bromine derivatives of the hydrocarbons of the aliphatic series, and acids and their oxygen derivatives, phenols, and hydrocarbons of the carbocylic series. The work is not well organized, and the results lack quantitative value. The extent of growth of the fungus in the different solutions is indicated by + and −. The ranges of concentrations of the substances used are not sufficiently wide to permit the determination of inhibiting concentrations with any degree of exactness. Moreover, the concentrations used are entirely arbitrary and without reference to chemical properties of the substances. In some cases of slightly soluble substances (the higher fatty acids) the concentrations were not known. Some of the results appear unusual. It is known that the various species of blue molds are not particularly selective as to their food. It is surprising, nevertheless, to note that such substances as chloroform, carbon-tetrachloride, dichlor-brom-ethylene, and benzene are said to serve as nutrients for these molds. The question occurs whether the belated growth in flasks containing these somewhat volatile substances was anything more than the film of growth formed by *Penicillium* even on the surface of inert solutions to which no organic nutrients have been intentionally added. The authors believe that a general parallelism exists between the relative toxicity of the various substances and their partition coefficient in oil and water. They find, therefore, a satisfactory explanation of the different behavior of the substances in the theories of MEYER and OVERTON, both of which, it should be stated, however, were formulated with reference to chemically inert substances or, at most, very weakly basic organic substances (OVERTON).

The conclusions reached by the authors may be summed up as follows. In general, substances act as food or poison according to the magnitude of their partition coefficients in oil and water, those relatively very soluble in oil being highly toxic, and those with a low partition coefficient serving as food. Some substances (cetyl alcohol, higher fatty acids, napthalene) very soluble in oil but only slightly soluble in water serve as food, and by reason of their slight

[6] BOESEKEN, J., and WATERMAN, H., Over een biochemische methode ter bepaling van kleine haeveelheden salicylzuur naast een overmaot p-oxy benzoëzuur. Konink. Akad. Wetensch. Amst. **20**[1]:548–552. 1911.

———, Over de werking van eenige benzolderivaten op de ontwikkeling van *Penicillium glaucum*. *Ibid.* 552–567. 1911.

———, Over de werking van eenige koolstofderivaten op de ontwikkeling van *Penicillium glaucum* en hunne remmende werking in verband met oplosbaarheid in water en in olie. *Ibid.* **20**[2]:965–973. 1912.

———, Werking van in water gemakkelijk, in olie niet oplosbare stoffen op den groei van den *Penicillium glaucum*. *Ibid.* 1246–1251. 1912.

solubility in water are not toxic. Substances easily soluble in water act either as poisons or as nutrients according to their relatively higher or lower solubility in oil. Substances easily soluble in water and slightly soluble in oil act as nutrients, but not as poisons. In other cases, where this theory fails to explain the toxicity of substances, as with some dibasic acids easily soluble in water and scarcely soluble in oil, which nevertheless are toxic, the toxicity is attributed to the hydrogen ion. These exceptions, as well as a number of others (formic acid, etc.), go to show that the action of substances on organisms cannot be explained on the basis of any one characteristic of the substances. The hydrogen ion is toxic, but acetic acid possesses a toxicity far in excess of that attributable to its hydrogen ion. Cane sugar, regardless of its solubility in oil or in water, can be utilized only by those organisms which contain invertase.

The inhibiting and sometimes fatal effects which the accumulated products of metabolism exert on organisms producing them are matters of general observation. Different organisms vary much, however, in their behavior toward their own products. An interesting illustration of this difference of behavior is brought out by WEHMER[7] in his studies of the effect on *Penicillium variabile* and *Aspergillus niger* of acids accumulating in culture solutions upon which these fungi are growing. The behavior of these two organisms differs widely. In cultures of *Penicillium* on solutions containing ammonium sulphate, WEHMER observed inhibition of growth and ultimately death of the fungus as a result of the accumulation of free acid in the solution. This result does not come about in cultures in which potassium nitrate, ammonium nitrate, ammonium chloride, or ammonium salts of organic acids are the source of nitrogen. In the case of nitrates, both ions are consumed, although here also nitric acid accumulates in the cultures at first. Hydrochloric acid seems to be comparatively harmless to this fungus. In cultures of *Aspergillus niger*, also, acid accumulates in the solution when ammonium salts of inorganic acids are offered as sources of nitrogen, but in the course of a few weeks there is in all cases a diminution of the acidity of the solution. The diminution is most marked with sulphuric acid and least with hydrochloric acid. Growth is not injured by the temporary accumulation of acid, but spore production is inhibited. The acidity is due to the accumulation of inorganic acid and not to the production of organic acids. The author attributes the lowering of the acidity of the cultures to a neutralization of the acid by the products of the protein decomposition in the older parts of the mycelium. Thus the ammonia consumed during the early growth of the culture is finally

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[7] WEHMER, C., Selbstvergiftung in *Penicillium*-Kulturen als Folge der Stickstoff. Ernährung. Ber. Deutsch. Bot. Gesells. **31**:210–225. 1913.

———, Der Gang der Acidität in Kulturen von *Aspergillus niger* bei wechselnder Stickstoffquelle. Biochem. Zeitschr. **59**:63–76. 1914.

liberated and becomes available for the neutralization of the accumulated acid when through want of a carbon nutrient rapid growth is no longer possible.

KIESEL[8] has examined a large number of acids, mostly organic, and salts of some inorganic acids with reference to their toxicity to *Aspergillus niger*. The numerous data which do not permit of any brief generalizations are, like other data of this kind, of interest more from a toxicological than from a physiological standpoint. The author's conclusion that the toxicity of organic acids cannot be entirely ascribed to the dissociated part of the molecule is in accordance with the findings of other investigators. The mode of action of the dissociated part of the molecule has not thus far been explained. The author's hypothesis, that toxic action which cannot be correlated with chemical properties is associated with the degree of permeability of the protoplasm to the substances exerting such action, is interesting in the light of the researches of MYER and of OVERTON, but it lacks experimental evidence, for it has not been shown on the one hand that toxicity and power to penetrate the protoplast go hand in hand in such cases, and on the other hand it is self-evident that substances which do penetrate the protoplast cannot act as poisons. The author fails to find a regulatory depression of acidity of the medium, as has been reported by some investigators. With regard to the relative toxicity of the chlor-acetic acids, KIESEL'S results harmonize with those of CLARK, who found that the introduction of one or two chlorine atoms into acetic acid increased its toxicity, but the introduction of a third chlorine atom lowered the toxicity.

The problem of antidotal action, or antagonism of one substance toward another, assumes special significance in the study of the toxicity of various substances to fungi, since the toxic substances whose effects are to be studied can rarely be used alone, but must usually be combined with other electrolytes or non-electrolytes requisite for the growth of mycelium and in most cases even for the germination of spores. Several papers dealing with this problem have appeared recently.

From this point of view BOESEKEN and WATERMAN[9] have studied the influence of a series of substances on the toxicity of boracic acid toward *Penicillium glaucum* and *Aspergillus niger*. The substances studied were glycerine, sorbite, dulcite, mannite, arabinose, xylose, glucose, levulose, mannose, rhamnose, galactose, maltose, lactose, raffinose, sucrose, p-oxybenzoic acid, protocatechuic acid, and gallic acid. Definite concentrations (usually 2 per cent) of these substances were used in connection with varying concentrations of the acid. The culture solutions were made with tap water, to which

[8] KIESEL, A., Recherches sur l'action de divers acides et sels acides sur le développement de l'*Aspergillus niger*. Ann. Inst. Pasteur **27**:391–420. 1913.

[9] BOESEKEN, J., and WATERMAN, H. J., Über die wirkung der Borsäure und einiger anderen Verbindungen auf die Entwicklung von *Penicillium glaucum* und *Aspergillus niger*. Fl. Microbiologica **1**:342–358. 1912.

ammonium chloride, potassium di-hydrogen phosphate, and magnesium sulphate had been added. The effect of the acid in combination with the different substances was estimated by the relative development of the cultures. It is noted that the antitoxic action of the substances examined is correlated with the ease with which they combine with the acid, as shown by conductivity changes of the mixtures. Those which combine most readily with boracic acid are also most effective as antidotes to the toxic action of the acid. The antagonistic action of these substances, therefore, is attributed to their power of holding the acid in combination.

Studies of a similar nature have been made by KUNKEL,[10] who investigated the antagonistic action with reference to *Monilia sitophila* between inorganic salts and such substances as peptone, starch, glucose, fructose, and galactose, which are frequent constituents of culture media. These substances all modify the toxicity of salts, but not to the same extent nor in the same order. The author believes that this modification of toxicity may in part be the result of reactions between the salts and the organic substances of the medium,[11] but there appears to be no constant correlation between reduction in the concentration of ions caused by the addition of nutritive substances and the toxicity of the medium. On this account the author believes that the food substances themselves have an influence on the organism by which it is enabled to endure higher concentrations of poisons. This conclusion could hold good only in cases where the toxic effect was entirely due to the ionized portion of the molecule, otherwise there would be no reason for the expectation of a relation between ionic concentration and toxicity. The author finds that spores are capable of remaining alive a long time, two weeks or more, in the presence of toxic substances, provided the concentration is below that at which plasmolysis occurs. It is also noted that slow growth occurs in toxic solutions slightly below the limit concentrations. As a possible explanation of the mode of action of toxic substances in such cases, the author suggests that the effect of the poisons is due to the hindrance of water-absorption by the protoplasm. This hypothesis, it will be remembered, was suggested long ago by LIVINGSTON,[12] who observed a similarity between the action of salts on the cells of the alga *Stigeoclonium* and the withdrawal of water from the cells. In conclusion, the author points out, with justice, that the composition of the medium should be taken into consideration in studies on toxicity of substances to plants.

[10] KUNKEL, L. O., The influence of starch, peptone, and sugars on the toxicity of various nitrates to *Monilia sitophila* (Mont.) Sacc. Bull. Torr. Bot. Club **40**:625–630. 1913.

———, Physical and chemical factors influencing the toxicity of inorganic salts to *Monilia sitophila* (Mont.) Sacc. *Ibid.* **41**:265–293. 1914.

[11] See foregoing review.

[12] LIVINGSTON, B. E., Chemical stimulation of a green alga. Bull. Torr. Bot. Club **32**:1–34. 1905.

LE RENARD[13] attempts to establish as a measure of the antitoxic action of a substance the "antitoxic coefficient," which is defined as the ratio between the number of liters containing a gram-molecule of the antitoxic substance in centinormal solution (that is, 100 in the case of monovalent substances) and the number of liters in which a gram-molecule of the toxic substance is dissolved at the limit concentration. The propositions which the author derives from the ratio thus defined, and with whose derivation the first part of the paper is concerned, all follow from the arithmetical relations of the quantities involved in the definition, and like the antitoxic coefficient itself have no physiological significance. In his experiments LE RENARD studied the antagonistic action with reference to *Penicillium glaucum* of formates, acetates, sulphates, and nitrates of potassium, ammonium, and magnesium, and the phosphates of potassium and ammonium in combination with salts (mostly chlorides and nitrates) of the heavy metals. The antitoxic solutions were used in concentrations ranging in a geometrical progression with a ratio of 1/10, usually from N 10^{-2} to N 10^{-5}. The toxic substance was generally used in one or two arbitrarily chosen concentrations. The results show an unusual regularity for biological data. The chief conclusion of the author is that the resistance of *Penicillium* to poisons varies according to the nutritive medium in one of the following ways: (1) the resistance varies, in a simple ratio, inversely as the concentration of the antitoxic substance; (2) the resistance passes a maximum at a comparatively low degree of concentration of the antitoxic substance; or (3) the resistance is not modified. The first part of this conclusion seems scarcely to be borne out by the data, for in nearly all cases where different concentrations of the toxic substances were used the quantities endured by the fungus fell with a diminution of the concentration of the antitoxic substances. It is probable, furthermore, that the use of a greater number of concentrations with smaller intervals between them would have led the author to modify his conclusion as to the simple relation said to exist between the concentration of an antitoxic substance and its effect on the toxicity of another substance. The recent work of SZÜCS[14] has clearly shown that the antagonistic ionic effects do not follow any such simple law of proportionality.

The antagonism between the nitrates of calcium, magnesium, and potassium on the one hand, and nitrates of copper, lead, zinc, aluminum, and nickel on the other hand, with reference to the spores of *Glomerella cingulata*, has been investigated by HAWKINS.[15] The technique employed was similar to

[13] LE RENARD, A., Influence du milieu sur la résistance du Pénicille crustacé aux substances toxiques. Ann. Sci. Nat. Bot. IX. **16**:277–336. 1912.

[14] SZÜCS, J., Experimentelle Beiträge zu einer Theorie der antagonistischen Ionenwirkungen. Jahrb. Wiss. Bot. **51**:85–142. 1913 (rev. in BOT. GAZ. **56**:85. 1913).

[15] HAWKINS, L. A., The influence of calcium, magnesium, and potassium nitrates upon the toxicity of certain heavy metals toward fungus spores. Physiol. Researches **1**:57–92. 1913.

that used and fully described by CLARK. HAWKINS finds in general that the addition of the nitrates of calcium, magnesium, and potassium to solutions of nitrates of copper, lead, or zinc reduces the toxicity of the solutions. With aluminium nitrate no reduction of the toxicity was observed. Nickel nitrate proved toxic only in high concentrations and was not used in combination with the antitoxic nitrates. For combinations of copper nitrate with calcium nitrate it is shown, by considerations based on the mutual influence of salts with a common ion upon the degree of dissociation of each other, and by potentiometer measurements of the concentration of copper ions in the mixture, that neither the reduction of the degree of ionization brought about by the mixing of the salts nor the formation of double salts will account for the lowering of the toxicity of the copper solution by the addition of the calcium salt. For combinations of lead nitrate with nitrates of calcium and magnesium, it is shown that reduction in ionization cannot account for the reduced toxicity of the lead salt. For lead nitrate and zinc nitrate within the limited range of concentrations used (3) there is a constant ratio between the molecular concentration of the toxic salt and that of the antitoxic salt necessary to inhibit the action of increasing concentrations of the toxic salt. For the combination of copper nitrate with calcium nitrate no such constant ratio was evident. The author concludes that the antagonistic action of one salt upon another cannot be attributed, as some investigators have done, either to reduction of the dissociation of the toxic salt or to the formation of undissociated double salts.

Incidental to an investigation, the object of which was to determine the factors inducing the formation of giant cells and mucor yeast by the mucors, RITTER[16] has reported a few experiments on the influence of nitrogenous compounds and sodium chloride on the toxicity of acids. The inhibition of germination of the spores was taken as a criterion of toxicity, although some difficulty was experienced with this test because many of the mucor spores began to form giant cells in concentrations of acid far below the toxic limit. The culture solutions contained, in addition to potassium di-hydrogen phosphate and magnesium sulphate, either peptone or ammonium nitrate and sugar. It was found that malic, citric, tartaric, nitric, and hydrochloric acids were much less toxic in the peptone medium than in the ammonium nitrate medium. The author generalizes from these observations to the effect that the toxicity of organic and inorganic acids is increased by the presence of inorganic nitrogen and depressed by organic nitrogen compounds. The data, it should be remarked, do indeed show an increased toxicity of acids in the presence of ammonium salts as compared with organic nitrogen compounds, but no data showing an absolute increase of toxicity as a result of the addition of ammonium

[16] RITTER, G. C., Die giftige und formative Wirkung der Säuren auf die Mucoraceen und ihre Beziehung zur Mucorhefebildung. Jahrb. Wiss. Bot. **52**:351–403. *pl. 1.* 1913.

salts are presented. For combinations of citric acid and sodium chloride it was found that for a wide range of concentrations, mixtures of these substances were more toxic than either alone. In this case the effect seems to be additive. The concentration of hydrogen ions was found to be the chief factor determining the production of giant cells. The production of mucor yeast, which is in no wise related to the production of giant cells, is determined chiefly by the absence of oxygen in slightly acid media containing sugar.—H. HASSELBRING.

Food substances and growth.—The fact that any given result in plant physiology is usually the result of several factors and is only rarely traceable to one factor alone receives further emphasis in the recent work of BOTTOMLY.[17] Mineral nutrients and toxins have received much attention in the discussion of the causes of soil fertility, and both have been shown to be limiting factors in certain cases. BOTTOMLY'S work emphasizes the idea that the soluble humus of the soil is an essential factor in soil fertility, providing not only food and energy for numerous soil bacteria, but also serving as a source of food for plants. His interpretation of the work reported in this paper is that the chief interest in it centers around the possibility that the nutrition of a plant depends, not only upon the supply of mineral food constituents, but also upon a supply of certain accessory organic food substances, very small amounts of which are sufficient to supply the needs of the plant. He cites literature indicating that other workers have found that soil humates stimulate the action of nitrogen-fixing bacteria and also that they can be readily assimilated by plants.

BOTTOMLY finds that when peat is submitted to the action of certain aerobic soil organisms (he does not say what ones) at 26° C., it decomposes rapidly "and a large amount of the humic acid present is converted into soluble ammonium humate." His use of the terms "humic acid" and "humates" is interesting in the light of the recent statement by SCHREINER[18] that "the compounds . . . . such as humic acid . . . . have absolutely no existence, but are shown to be mixtures of many widely different compounds." In this connection it may be noted that WIELER[19] has taken the view that "humic acids" in soils are inorganic acids resulting, for example, from the action of bases on salts; and that BAUMANN and GULLY[20] have shown that in peat soils the acid properties are due to the colloidal matter of the cell covering the hyaline sphagnum cells.

BOTTOMLY found that bacterized peat, after sterilization, was an excellent medium in which to grow nitrogen-fixing bacteria and apply them to the soil.

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[17] BOTTOMLY, W. B., The significance of certain food substances for plant growth. Ann. Botany **26**:531–450. 1914.

[18] SCHREINER, O., The organic constituents of soils. Science N.S. **36**:577–587. 1912.

[19] WIELER, A., Pflanzenwachstum und Kalkmangel im Boden. 8vo. pp. vii+235. *figs. 43*. 1912.

[20] BAUMANN and GULLY, quoted in Science N.S. **40**:492. 1914.

A large increase in the nitrogen content of soils resulted from the addition of active bacterized peat as compared with controls of the same soils with sterile peat. It was found that an aqueous extract of bacterized peat supplied all of the plant food necessary for water cultures of tomato, barley, and buckwheat seedlings. It was also found that bacterized peat contains a substance or substances that stimulate growth and enables the plants to utilize the normal mineral food constituents (NH_3, P_2O_5, and K_2O) more readily. It is supposed that in nature these growth-stimulating substances are supplied by the decayed organic matter of the soil. Experiments under way are reported to indicate that during the early stages of the growth of the embryo these substances are supplied from the seed.

The results of these experiments with bacterized peat coordinate well with agricultural practice as observed by the reviewer in the Puget Sound region of the United States. In this region sphagnum bogs are readily converted into productive gardens by drainage and cultivation. This growth-stimulating substance (or substances) is soluble in water and in alcohol and is precipitated by phosphotungstic acid. Very little has been determined as to the nature or composition of these growth-stimulating substances, but they are said to resemble in certain ways the accessory food bodies concerned in animal nutrition—GEORGE B. RIGG.

Some Ontario forest conditions.—In order to obtain some exact data regarding the extent and conditions of their forests, the Commission of Conservation of Canada has had surveyed among other regions a portion of Ontario east of Georgian Bay and north of Lake Ontario. The area is within the basin of the Trent River and comprises some 1,345,000 acres, slightly rolling in character, with a very thin soil over the recently glaciated granitic rocks of Archaean and Ordovician age. HOWE[21] reports that two-thirds of this area was originally covered with a more or less pure white pine forest, the remainder being chiefly of hard wood type, in which beech and maple predominated. Now the virgin forest is practically gone, although on account of the poor quality of the scanty soil less than 12 per cent of the area has been farmed and little more is tillable.

In discussing present conditions, four types of forest are recognized. (1) The pure coniferous forest with less than 10 per cent of other trees is made up of *Pinus Strobus* with a small quantity of *Tsuga canadensis*. It occupies less than 5 per cent of the woodland and is now hardly known in virgin condition. (2) The pure hard wood forest contains less than 10 per cent of coniferous trees and occupies the deeper soils, covering about 33 per cent of the forested area. It is composed of *Acer saccharum*, *Fagus grandifolia*, *Betula lutea*, *Tilia americana*, and a few other minor species. From the predominance of the two species first named, both as mature trees and as seedlings, it is evident that this

[21] HOWE, C. D., and WHITE, J. H., Trent watershed survey. Commission of Conservation, Canada. pp. 156. *ills.* 16. *maps* 3. 1913.

represents the climax forest of the region. (3) Then comes a mixed forest occupying only some 6 per cent of the wooded area and made up partly of a combination of the previously mentioned types and partly of a swamp type in which *Fraxinus nigra, Thuja occidentalis,* and *Abies balsamea* are dominant. (4) Finally, there are areas formerly mostly pine forests, but repeatedly burned after cutting and now occupied by a pioneer association dominated by *Populus tremuloides* and *Betula alba.* It comprises some 56 per cent of the forested area, occupying the thin soils over the granitic or crystalline rocks or the deeper sandy plains and sandy ridges. While potentially pine forest areas, these poplar-birch forests are usually so entirely without pine that only by a system of planting could they be brought to their original richly productive condition.

HOWE reviews at some length the economic loss involved in the forest fires so prevalent in the past and still occurring annually over this region, and shows the true economy of the preventive measures he recommends.

A discussion of the economic and industrial conditions by WHITE and an introduction by FERNOW both show the futility of attempting agriculture in a region so little suited to crop production, and the great importance of having it organized into a forest reserve under government control with scientific supervision.

The illustrations, the excellent index, and the mapping of the distribution of the forest types described all add to the value of the report.—GEO. D. FULLER.

Paleobotanical notes.—SEWARD[22] has published an account of the antarctic ("Terra nova") fossil plants collected by the British Antarctic Expedition of 1910, being the first of the geological memoirs completed. A general account of the various expeditions to this region is given, followed by a description of the paleobotanical material secured, much of it being too fragmentary for certain identification. Among the descriptions are two new genera, obtained from what are probably Mesozoic beds: *Antarcticoxylon* (presumably the stem of a gymnosperm) and *Pityosporites* (thought to be a winged pollen grain of some gymnosperm). Various remains of *Glossopteris* were also identified, and the occurrence of this genus in the antarctic regions suggests a general discussion of the wide uniformity of climatic conditions during the later Paleozoic.

SEWARD,[23] in another paper discussing the Wealden floras, calls attention to the surprising similarity in the general appearance of the floras of Japan, South Africa, North America, South America, Europe, and the Arctic regions. "In the Wealden period the type of vegetation was very similar to that which flourished through the greater part of the world during the whole of the Jurassic,

[22] SEWARD, A. C., Antarctic fossil plants. British Museum, Brit. Antarctic Exped. 1910. Geol. 1:1–49. *pls. 1–8.* 1914.

[23] ———, Wealden floras. Hastings and East Sussex Nat. 2:126–142. *pl. 2.* 1914.

and very shortly after the Wealden the vegetation of the world experienced a very remarkable transformation."

WIELAND[24] has investigated the problematical fossil *Cryptozoon*, and the much discussed question of the origin of the oolites. Oolites and *Cryptozoon* are said to be notable features of the Ozarkian. According to WIELAND, *Cryptozoon* is a marine alga "which formed vast reefs in the Ozarkian oceans"; and in connection with a description of a new species of *Cryptozoon* from Pennsylvania, and the general occurrence of similar forms (as *Eozoon*, for example) in the early Paleozoic, he concludes that the hypothetical "age of seaweeds" preceding the coal plants is a reality.

BERRY[25] has contrasted the ancestry of our present walnuts and hickories, so far as they can be recognized as fossils, back to the Middle Cretaceous, and presents evidence that at this remote period their geographical range and their abundance were much greater than now. This evidence also enables him to explain the geographical distribution of the living representatives of the family.—J. M. C.

Slope-direction and forest distribution.—TURESSON[26] points out that *Pseudotsuga taxifolia* (Douglas spruce or red fir) is confined to north-facing slopes in the Spokane region in eastern Washington. He says "the evidences have shown that exposure is the regulating factor in the distribution of the tree in this region, the northern slopes and ridges being the only localities which offer the needed humidity in soil and atmosphere." He adds "not only around Spokane but in all more or less arid regions can this be observed." He cites from his own observations and from literature several instances illustrating the fact that the southern slope tends to be more xerophytic than the northern. After calling attention to the fact that this tree reaches its best development in the Puget Sound region, he cites COWLES[27] to indicate that near its areal limits a species "can grow only in those formations which resemble most closely in an edaphic way the climatic features at the distribution center." Speaking of the distribution of this tree in the San Juan Islands, he calls attention to the similarity in climate between these islands and the Spokane region. He then says "it is not surprising to find *Pseudotsuga taxifolia* confined to the northern slopes of the hills in these islands." Quoting from a paper by the reviewer,[28]

[24] WIELAND, G. R., Further notes on Ozarkian seaweeds and oolites. Bull. Amer. Mus. Nat. Hist. **33**:237–360. *pls. 14–19.* 1914.

[25] BERRY, EDWARD W., Notes on the geological history of the walnuts and hickories. Smithsonian Report for 1913. pp. 319–331. 1914.

[26] TURESSON, G., Slope exposure as a factor in the distribution of *Pseudotsuga taxifolia* in eastern Washington. Bull. Torr. Bot. Club **41**:337–345. 1914.

[27] COWLES, H. C., The physiographic ecology of Chicago and vicinity. BOT. GAZ. **31**:73–108, 145–182. 1901.

[28] RIGG, G. B., Forest distribution in the San Juan Islands: a preliminary note. Plant World **16**:177–182. 1913.

he says, "RIGG has . . . . pointed out the seemingly peculiar distribution of *Pseudotsuga taxifolia* as limited to the north-facing slopes of the hills." It is perhaps generalizing too much to say that the species under discussion is confined to the north-facing slopes "in *all* more or less arid regions," although the phenomenon is undoubtedly of frequent occurrence. If the reviewer interprets COWLES correctly, the absolutely rigid application of the principle quoted from his paper of 1901 is not in accord with the spirit of his more recent teaching. In regard to the quotation from the reviewer's paper, the facts are that the paper cited pointed out *four cases only* in these islands where the forest is largely limited to the north-facing slopes. It would be generalizing too much to say that this is true in all cases in the islands. The whole subject of forest distribution in the San Juan Islands should be made the subject of a careful field investigation. The point of view from which the paper is written is very suggestive and it forms a valuable contribution to the subject of forest distribution in the Northwest.—GEORGE B. RIGG.

Available soil moisture.—ALWAY[29] has grown plants in water tight cylinders until they die from lack of available moisture, and made careful determinations of the moisture conditions of the soils. He concludes that for comparing the available moisture in soils either the wilting coefficient or the hygroscopic coefficient may be used with equal efficiency. The former seems to him preferable in considering conditions of germination and growth of crop plants, and the latter in considering the seed production of such annual plants as field grains and the maintenance of life of perennial plants. Under the conditions of his experiments, most plants seemed capable of producing little or no growth after the soil moisture fell below the wilting coefficient, but whenever there was a well developed root system and no remarkably unfavorable conditions obtained, the plants were able to reduce the moisture content of the soil almost or quite to the hygroscopic coefficient, that is, to 68 per cent of the wilting coefficient. Little difference was found between the ability of the various crop plants used in the experiment to exhaust the soil moisture; while, on the contrary, marked differences were evident in their ability to remain alive after showing injury from drought. Desert legumes of perennial habit remained alive after the water content of the soil had fallen distinctly below the hygroscopic coefficient, showing that the water taken by the soil from a saturated atmosphere may be to some extent available for the maintenance of the life of such plants, although it is evidently beyond the reach of ordinary crop plants. Incidentally, evidence is presented that in many soils of dry lands, the loss of water from all but the thin upper stratum takes places entirely through transpiration.

The bulletin reports a good example of an investigation in a quantitative manner of problems in plant production important alike to agriculture and ecology.—GEO. D. FULLER.

[29] ALWAY, F. J., Studies on the relation of the non-available water of the soil to the hygroscopic coefficient. Agric. Exp. Sta. Neb. Research Bull. **3**:133. *figs.* 37. 1913.

Tree growth.—DOUGLASS[30] hopes to find in tree growth an indicator that may be used for estimating rainfall, but the preliminary steps of his investigation are similar to those of botanical observers. Studying *Pinus ponderosa* grown in the northern plateau of Arizona, a semi-arid region where the amount of precipitation is almost certain to be the limiting factor of annual growth, his conclusions agree with those of KIRKWOOD in indicating the importance of the precipitation of the fall and winter months upon the amount of the increment of the succeeding growing season. Further, a most satisfactory explanation of double annual rings is found in the failure of fall and winter precipitation when the resulting spring drought is followed by the usual heavier rainfall of July and August. Sometimes when this drought is excessive, the later rains do not seem to be able to stimulate a late summer growth, and a very narrow single ring results. In a few instances DOUGLASS thinks that, for some unknown reason, there has been the entire suppression of one annual ring.

From measurements of annual rings, given in detail in a previous paper,[31] a growth record is obtained for the past five centuries. When this is plotted as a curve and a comparison made between an available rainfall record for the region extending back to 1867 and the portion of the growth curve for the corresponding period, there is found an agreement of 80 per cent, but we are warned that such an agreement is likely to obtain only for a dry climate. An effort to discover a regular periodicity in the growth rate is rather unsuccessful, although there seems to be some agreement with the sun-spot cycle of 11.4 years.—GEO. D. FULLER.

A hydrarch succession.—MATTHEWS[32] has reported on the study of the succession of plant associations occurring in the gradual filling up of a pond of some 16 acres in area situated in Perthshire, Scotland. Aquatic and marsh associations of the usual type are found, but with an unusual paucity of species. —GEO. D. FULLER.

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[30] DOUGLASS, A. E., A method of estimating rainfall by the growth of trees. Bull. Amer. Geogr. Soc. **46**:321–335. 1914.

[31] ——— Weather cycles in the growth of big trees. Month. Weather Rev. **37**:225–237. 1909.

[32] MATTHEWS, J. R., The White Moss Loch: a study in biotic succession. New Phytol. **13**:134–148. *figs. 2*. 1914.